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TOCOPHEROL CONTENT OF SEAWEED AND SEAWEED MEAL

I.—Analytical methods and distribution of tocopherols in benthic algae

By A. JENSEN

A rapid and reliable method for identification and quantitative determination of algal tocopherols has been developed. The algal tocopherols are separated from each other and from other lipids by circular chromatography on paper which contains aluminium oxide. Based on this method the distribution of tocopherols in brown, red and green seaweeds was investigated. α-Tocopherol was the only tocopherol detectable in all algae except for those belonging to the Fucaceae family, which contained the γ- and δ-homologues as well.

Introduction

In 1953 Brown[1] demonstrated the presence of considerable quantities of tocopherols in marine algae. This led to the generally accepted assumption that seaweed meal is a good source of vitamin E, although no determination of the tocopherol content in this product has been published, and the effect of commercial drying and storage is not known. In fact the algal material studied by Brown had been dried in the laboratory and stored for undefined periods prior to analysis, and his results may not be representative of the algae as such. Very recently Skinner & Sturm[2] published data on the content of α-tocopherol in some seaweeds. Their results deviated considerably from those obtained by Brown.

The increasing use of seaweed meal as a feed ingredient, and the change from drying on the rock in summer to year-round production by artificial drying have made it even more important to establish the seasonal variation and the influence of processing on the content of tocopherols in seaweed meal. Furthermore it was of considerable importance to study the distribution of tocopherols in a larger number of marine algae, and to obtain a more rigid identification of the tocopherols found by Brown.

The present paper deals with the development of a rapid and reliable method for the identification and quantitative determination of algal tocopherols and with studies on the distribution of tocopherols in Norwegian seaweeds.

Experimental

The reagents used in this and the later studies[3,4] were those recommended by the Analytical Methods Committee[5] except that methanol (Methanol, reinst, E. Merck & Co., Darmstadt) was used in the ferric chloride and the dipyridyl reagents, instead of ethanol.

The chromatographic paper was supplied by Schleicher & Schüll A.-G., Dassel, West Germany; it was originally described as No. 667, but this was later changed to No. 288. Both brands were found acceptable, and they contained approximately 20% Al_2O_3. The papers were either used directly without activation, or they were dipped in a solution (0·02% wt./vol.) of fluorescein (May & Baker, Ltd., England) in ethanol, excess solution being pressed off between filter papers, and the treated papers being dried at 40° over-night.

The algal materials were collected in the Trondheimsfjord during winter and early spring. Only healthy plants free of parasitic and saprophytic contamination were used. The material was brought to the laboratory and worked up immediately in most cases.

A 3 g sample of the alga was ground with successive portions of acetone in a mortar until the solvent extracted no more pigmented material. The combined extracts were filtered through a G1-sintered glass filter and concentrated to dryness *in vacuo* at room temperature. The residue was taken up in a small volume of acetone and the solvent was again removed *in vacuo* at room temperature. The lipids were then extracted into acetone–petroleum ether (1:1 by vol.) and the volume of the extract was adjusted to 2 ml.

Measured samples of 0·1—0·4 ml were applied to the centre of the 18 cm chromatographic paper as described earlier for chloroplast pigments.[6] The chromatograms were run in Petri dishes covered with a glass plate, with diethyl ether (20–25% by vol.) in petroleum ether as the irrigant. Untreated papers were used for visual inspection of the tocopherols which were made visible by spraying the chromatogram with Emmerie–Engel[7] reagent. Fluorescein-treated papers were viewed in filtered ultra-violet light, and the zones of quenched fluorescence were marked with a pencil. The corresponding rings were cut out, packed tightly into 8 mm (i.d.) glass tubes which were drawn out to a capillary at one end, and the tocopherol was eluted into a 5 ml volumetric flask with 2 ml methanol followed by 2 ml benzene. To this mixture 0·2 ml of the methanolic dipyridyl solution was added and the bottle was filled up to the mark with methanol. A ring of the paper was cut from the chromatogram outside the solvent front, extracted in the same way and used as a blank.

To each of the extracts and the blank was now added 0·2 ml of the methanolic ferric chloride solution, and the colour formed was measured against the blank at 520 nm after exactly 5 min. The calculation of the tocopherol content of the extracts was based on the extinction values given in the literature.[5]

The three, separate, tocopherol extracts obtained from paper chromatograms of *Ascophyllum nodosum* and *Pelvetia canaliculata* were concentrated to small volumes and subjected to two-dimensional thin-layer chromatography on silica gel (Riedel-de Häen, DC-Karten S 1) using chloroform for the first development and di-isopropyl ether (20% by vol.) in petroleum ether for the second.

The separated tocopherol extracts obtained from two paper chromatograms of *A. nodosum* and *P. canaliculata* were con-

centrated to dryness *in vacuo* at room temperature and taken up in carbon disulphide (20 μl), and aliquots (3 μl) were injected into the port of an Aerograph 300 gas chromatograph. The column used was a 6 ft stainless-steel column ($\frac{1}{8}$ in. o.d), filled with Chromosorb W (100/120 mesh) containing SE-30 (2% by wt.). The injector and the detector were operated at 265° and the column at 253°. Gas flow rates were 34 ml/min for the carrier gas (nitrogen), 40 ml/min for the hydrogen and approximately 300 ml/min for the purge gas. For comparison the tocopherols of unrefined soyabean oil, isolated by paper chromatography, were subjected to gas/liquid separation under identical conditions. Retention times for authentic α-tocopheryl acetate (Fluka) and for saponified α-tocopheryl acetate were also determined in separate runs.

Results

Identification of tocopherols by circular chromatography on alumina-containing paper

After development in the chromatographic system, the unsaponifiable fraction of soyabean oil was revealed as three red rings by Emmerie–Engel reagent.[7] Soyabean oil is known to contain α-, γ- and δ-tocopherol.[8,9] Application directly on to the chromatograms without prior saponification gave the same three zones. The total lipids present did not interfere with the chromatographic separation. The tocopherols showed up as dark rings when fluorescein-treated chromatograms were viewed in filtered ultra-violet light. Authentic α-tocopherol, obtained by saponification of commercial α-tocopheryl acetate (vitamin E acetate, Fluka), co-chromatographed with the outer zone of the soyabean tocopherols.

The best separation was achieved without activation of the papers prior to chromatography. Since the humidity of the air in the laboratory varied somewhat with season, the activity of the chromatographic papers also varied, showing higher activity in winter than in summer. Although this was partly compensated by regulating the content of diethyl ether in the solvent system, the R_f values varied somewhat from time to time. Typical values obtained are given in Table I and show that a satisfactory separation of α-, γ- and δ-tocopherol was obtained.

Wheat-germ oil gave a typical α-zone and a double ring with a R_f value of 0·50 – 0·58. According to Green *et al.*[9] and Mason & Jones,[10] wheat-germ oil contains α- and β-tocopherol and β-tocotrienol. Chromatograms of the lipid fraction of barley also showed two zones giving a positive Emmerie–Engel reaction. The strong outer zone was double and probably contained α-tocopherol and α-tocotrienol, while the inner zone was not resolved and consisted mainly of β-tocotrienol.[9,10] Wheat-bran lipids gave two double zones upon paper chromatography; the outer was taken to contain α-tocopherol and α-tocotrienol, and the inner one probably contained β-tocopherol and β-tocotrienol.[9,10] It should be noticed that all the actual lipid extracts were applied directly on to the paper. No saponification was needed.

When lipid fractions extracted from seaweeds belonging to the Fucaceae were similarly examined, three clear zones could generally be detected with Emmerie–Engel reagent. These co-chromatographed exactly with the tocopherols of soyabean oil. Comparison of algal tocopherols with soyabean tocopherols and with authentic α-tocopherol was also carried out by thin-layer chromatography using the two-dimensional system of Whittle & Pennock.[8] In all cases the algal and corresponding soyabean homologues co-chromatographed exactly. In addition the γ- and δ-compounds from algal sources reacted with diazotised *o*-dianisidine[5] to give blue–green and reddish–brown zones, respectively, while the α-homologue did not give any colour with this reagent.

The β-tocopherol fraction from wheat-germ oil was isolated by chromatography on alumina and compared with the corresponding compound isolated in the same way from an extract of *P. canaliculata*. The two isolates co-chromatographed on paper and on thin-layer plates using the systems of Whittle & Pennock,[8] of Pennock *et al.*[11] and of Stowe.[12]

The mixture of tocopherols obtained from paper chromatograms of extracts of *A. nodosum* and of *P. canaliculata* gave three tocopherol peaks in the gas chromatograph.[13] The retention times observed for tocopherols of algal and other origin are given in Table II.

Distribution of tocopherols in some marine algae

By using the paper chromatographic system, twenty-five different species of marine algae were investigated for their composition of tocopherols, and the results are collected in Table III.

All the algae investigated contained α-tocopherol, whereas only the Fucaceae showed the α-, γ-, δ-distribution pattern detected by Brown.[1] The latter algae also contained small quantities of an additional tocopherol which co-chromatographed with β-tocopherol from wheat-germ oil both on paper and thin-layer chromatograms.

TABLE I

R_f values of some tocopherols

Compound	R_f value (×100)
α-Tocopherol	62
α-Tocotrienol	58*
β-Tocopherol	50*
β-Tocotrienol	46*
γ-Tocopherol	37
δ-Tocopherol	25

*Tentative identity

TABLE II

Retention times of tocopherols

Compound	Source	Retention time
δ-Tocopherol	Soyabean oil	4 min 45 sec
γ-Tocopherol	Soyabean oil	6 min 7 sec
α-Tocopherol	Soyabean oil	7 min 50 sec
δ-Tocopherol	*P. canaliculata*	4 min 45 sec
γ-Tocopherol	*P. canaliculata*	6 min 5 sec
α-Tocopherol	*P. canaliculata*	7 min 51 sec
δ-Tocopherol	*A. nodosum*	4 min 45 sec
γ-Tocopherol	*A. nodosum*	6 min 5 sec
α-Tocopherol	*A. nodosum*	7 min 45 sec
α-Tocopherol acetate	Fluka	9 min 7 sec
α-Tocopherol	Fluka	7 min 40 sec

TABLE III

Distribution of tocopherols in some seaweeds

Species	Tocopherol			
	α	β	γ	δ
Pylaiella litoralis	+++			
Ectocarpus sp.	+++			
Dictyosiphon foeniculaceus	+++			
Laminaria digitata, fronds	+++			
Laminaria hyperborea, fronds	+++			
Laminaria saccharina, fronds	+++			
Alaria esculenta	+++			
Halidrys siliquosa	+++			
Himanthalia lorea, fronds	+++			
Himanthalia lorea, receptacles	+++			
Fucus serratus	++	+	++	++
Fucus spiralis	++	+	++	++
Fucus vesiculosus	++	+	++	++
Pelvetia canaliculata	++	+	++	++
Ascophyllum nodosum	++	+	++	++
Desmarestia aculeata	+++			
Dumontia incrassata	+++			
Furcellaria fastigiata	+++			
Gigartina stellata	+++			
Odonthalia dentata	+++			
Polysiphonia fastigiata	+++			
Porphyra umbilicalis	?			
Rhodomela subfusca	+++			
Rhodymenia palmata	+++			
Enteromorpha intestinalis	+++			
Ulva lactuca	+++			

+++ Only or predominant tocopherol present
++ Important component
+ Minor tocopherol

Quantitative chromatography of tocopherols by circular chromatography on alumina paper

In order to investigate the suitability of this special method for the quantitative determination of tocopherols, known amounts of α-tocopherol were subjected to chromatography on papers pre-treated with fluorescein solution. The u.v.-quenching zone was cut out, eluted with methanol and benzene and the concentration of α-tocopherol in the eluate determined by the Emmerie–Engel reaction. Parallel aliquots were diluted with solvent and treated directly with Emmerie–Engel reagent. The concentration of α-tocopherol in the original solution was determined by u.v. spectroscopy. The results are given in Table IV, from which it is seen that the recovery of α-tocopherol from the chromatograms was satisfactory. Although they were not determined it was expected that the recoveries of the other tocopherols would be similar, since Whittle & Pennock[8] have reported little or no difference in recovery for the different tocopherols. They reported percentage recoveries between 91 and 93 for the various tocopherols after two-dimensional thin-layer chromatography.

The tocopherol content was determined by the above method for a number of seaweeds. The results are given in Table V.

TABLE IV

Percentage recovery of α-tocopherol from circular paper chromatography

Quantity applied, μg	Recovery, %			Average, %
17·9	90·0;	91·5;	92·4; 94·0	92·0
107·4	97·2;	97·3;	99·9; 100·0	98·6

TABLE V

Tocopherol content of some seaweeds

Species	Tocopherols, mg/kg of dry matter			
	α	β+γ	δ	Total
*Alaria esculenta**	30			30
*Laminaria digitata**	9			9
*Laminaria hyperborea**	10			10
*Laminaria saccharina**	7			7
Ascophyllum nodosum	80–160	60–100	110–250	250–510
Fucus serratus	120–200	60–150	100–250	300–600
*Fucus spiralis**	138	70	148	356
Fucus vesiculosus	100–170	40–100	100–200	250–480
Pelvetia canaliculata	150–220	70–140	120–320	350–650
*Furcellaria fastigiata**	17			17
*Gigartina stellata**	35			35
*Polysiphonia fastigiata**	80			80
*Rhodymenia palmata**	35			35
Porphyra umbilicalis	<10			<10
Odonthalia dentata	20			20
Rhodomela subfusca	34			34
*Enteromorpha intestinalis**	92			92
*Ulva lactuca**	35			35

*Spring samples

Discussion

Analytical method

Until the present time, eight different compounds belonging to the vitamin E group have been found in nature.[11] These are the α-, β-, γ-, and δ-tocopherols and the corresponding tocotrienols. Of these only three have been detected in seaweeds, namely the trimethyl tocol (α-tocopherol), one of the dimethyl tocols (γ-tocopherol) and the monomethyl tocol (δ-tocopherol). Consequently a number of chromatographic methods are available for their separation. The identification of individual tocopherols has recently been much improved by the use of thin-layer chromatography.[8,11,12,14,15] In combination with specific staining and catalytic hydrogenation, thin-layer chromatography offers a reliable identification of all the naturally occurring tocopherols.

However, thin-layer chromatography has certain disadvantages in the quantitative determination of particularly labile compounds. There is always a serious risk of oxidation on the highly exposed surface of a thin-layer chromatogram, and in addition quantitative removal of the tocopherol spots and complete extraction of the material adsorbed are problematic. It must be admitted that good recoveries of tocopherols have recently been reported in two-dimensional thin-layer chromatography by, among others, Whittle & Pennock.[8] Nevertheless, paper chromatograms are more easily handled, and the method most frequently used for the quantitative determination of individual tocopherols is the two-dimensional paper chromatographic technique of Green *et al.*[9] slightly modified by Mason & Jones[10] and by Booth[16] and recommended by the Analytical Methods Committee.[5] This is still quite a laborious and difficult method, not very well suited for numerous routine determinations. One of the problems is the preparation of the chromatographic paper for reverse-phase separation. This operation is carried out with the tocopherols already spread out on the paper. In addition the

two-dimensional development takes several hours, and is interrupted by an undesirable drying operation. The reverse-phase separation is also easily disturbed by the presence of various lipids, and a saponification step or some other introductory purification is therefore required. Losses of tocopherols are invariably encountered during saponification[13] since the tocopherols are very labile in alkaline media. A further complication is introduced by the fact that the β- and γ-tocopherols are not separated by Green's method.

Since at this laboratory there was considerable experience with chromatography on alumina-containing papers for the separation of chloroplast pigments,[17,18] and since Lichtenthaler's work[19] had indicated the usefulness of this type of paper for the separation of plant quinones, an attempt was made to develop a quantitative method for the determination of tocopherols based on circular chromatography on alumina paper. A preliminary report on the applicability of this method has been published.[20]

The conclusion to be drawn from the paper chromatographic separations of grain and soyabean-oil tocopherols carried out in the present work is that the tocopherols reported to occur in marine algae should be easy to separate by this technique. Additional tocopherols should be detectable on the paper chromatograms as double zones, if such tocopherols occurred in the material. Thin-layer chromatography would then be needed to establish the identity of additional components.

The occurrence of three Emmerie–Engel positive main zones on paper chromatograms of the lipid extracts of the Fucaceae investigated, and the co-chromatography of the tocopherols involved either with authentic α-tocopherol or with corresponding tocopherols isolated from soyabean oil proved the applicability of the paper chromatographic method for the separation of algal tocopherols.

Recovery of α-tocopherol after paper chromatographic separation according to the new method was satisfactory and demonstrated the applicability of the procedure to quantitative studies. The method is simple and rapid, taking one or two hours including extraction of the plant material, chromatographic separation, extraction of the components and colorimetric determination. The chromatographic separation itself takes approximately 20 minutes and the method is therefore well suited for routine determinations of algal tocopherols. In most cases no saponification is needed, but when tocopherol esters are present or when large amounts of saponifiable lipids obscure the chromatograms, saponification cannot be avoided.

Distribution of tocopherols in benthic algae

It must be admitted that the identification of the algal tocopherols still rests mainly on chromatographic evidence. However, co-chromatography both by paper, thin-layer and gas/liquid chromatography with either authentic (α-tocopherol) or native (γ- and δ-tocopherols) compounds has now been carried out and has made it unlikely that the algal tocopherols differ from the corresponding test compounds. Concerning the β-like homologue, which occurred in trace amounts in the Fucaceae, further evidence for its identity is needed.

As is evident from Table III, α-tocopherol is generally present in all the seaweed classes investigated. In general the red, green and sublittoral brown algae seem to be rather poor in vitamin E. The Fucaceae hold a very special position among the seven algal families investigated, because of their relatively high content of α-tocopherol and especially because they contain the γ- and δ-homologues in addition to the α-compound. It is worth noticing that both *Himanthalia lorea* and *Halidrys siliquosa*, which belong to families (Himanthaleaceae and Cystoceiraceae, respectively) of the same order as do the Fucaceae, contain only α-tocopherol. This demonstrates the unique position held by the members of the Fucaceae family. It is possible that this influences their ability to survive desiccation in the intertidal zone, where they abound. However, other algae which contain only α-tocopherol also thrive in the same zone.

In general, the seaweeds investigated were poor sources of vitamin E, except for the Fucaceae. Various samples of green leaves have been reported by Booth[21] to contain 120–250 ppm of α-tocopherol, and Bunnel *et al.*[22] found 30–140 ppm of α-tocopherol in dehydrated lucerne. The green alga *Ulva lactuca* (Table V) was rather poor in this connexion.

The contents of tocopherols given in Table V are generally somewhat higher than the values reported by Brown for the same algae, and the relative proportion of α-tocopherol in the Fucaceae was consistently found to be higher than that previously reported. It was noticed that saponification tends to increase the relative amount of δ-tocopherol, and it has also been found that tocopherols are slowly lost during drying and storage of seaweed samples.[4] This, in addition to geographic, seasonal and individual variation between the different algal samples studied by Brown and in this laboratory, can explain the differences observed.

The apparent lack of α-tocopherol in one green and two red seaweeds and the low content in a *Fucus* species reported by Skinner & Sturm[2] seem more difficult to understand in the light of the present findings. It is possible that a considerable part of the original tocopherol content was lost during the rather long and extended analytical procedure followed by Skinner & Sturm.

All chromatograms of the algal extracts contained several zones in addition to the tocopherol rings. Several rings occurred invariably between the solvent front and the tocopherol zone. In some cases they contained compounds which could be saponified to give small quantities of tocopherols. From an extract of a spring sample of *A. nodosum*, a mixture of α-, γ- and δ-tocopherol corresponding to 2% of the total free tocopherols was obtained by saponification and chromatography of the outer zones of the original chromatogram. This indicated that a small part of the tocopherols in *A. nodosum* was esterified at certain times of the year. According to Dunphy *et al.*,[25] tocopherol esters occur in several species of land plants.

Additional outer rings of the chromatograms were not affected by saponification. They resembled the 'dimers' found on two-dimensional chromatograms of palm-oil lipids by Whittle & Pennock.[8] Oxidative dimerisation of α-, γ- and δ-tocopherols (from *Fucus serratus*) with *p*-benzoquinone as described by Nilsson *et al.*[23] gave products with co-chromatographed with several of the outer rings of the chromatograms of lipids of *Fucus* species. In addition, the synthetic dimers also reacted slowly with Emmerie–Engel reagent, giving the same reddish-yellow colour as did the 'natural dimers'. They all quenched the fluorescence of fluorescein-treated papers in u.v. light.

Another series of zones which migrated more slowly than that of δ-tocopherol occurred on many chromatograms. One of these, obtained from two paper chromatograms of *A. nodosum* lipids, gave the typical absorption spectrum of α-tocopherylquinone[23] in the ultra-violet (λ_{max} 260–270 nm in

methanol), oxidised leucomethylene blue, and co-chromatographed on paper with authentic α-tocopherylquinone prepared by iodine-catalysed oxidation of α-tocopherol.[23]

Although zones in both the 'dimer' and the 'tocopherylquinone' areas invariably were detected in chromatograms of extracts of fresh algae, their natural occurrence in these plants could not be safely established by the technique used, because of the many and partly overlapping zones that were seen in these areas of the chromatograms.

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TOCOPHEROL CONTENT OF SEAWEED AND SEAWEED MEAL

II.*—Individual, diurnal and seasonal variations in some Fucaceae

By A. JENSEN

The seasonal variation in tocopherol content in four brown seaweeds was followed through a two-year period. Considerable variation especially in the concentration of δ-tocopherol was established. The higher concentrations were found during autumn and winter, and low contents in spring. Individual and diurnal variations were also studied. Considerable differences in tocopherol content between various types of tissue were established. Surprisingly high concentrations of tocopherols were found in the older parts of the plants.

Introduction

In Part I[1] a rapid and reliable method for the quantitative determination of algal tocopherols was described. The distribution of individual tocopherols in a number of seaweeds was established, and the concentrations of these compounds were determined for spring samples of 26 species. It was pointed out that knowledge of the seasonal variation in the content of vitamin E was desirable for both scientific and practical reasons. Some of the Fucaceae are used in the seaweed-meal industry which now operates for almost the whole year. Seasonal variation in the raw material will therefore necessarily show up in the product. In addition, the content of α-, γ- and δ-tocopherols in the Fucaceae was found to be considerable, and more information related to the biological role of these compounds is needed. The other seaweeds investigated exhibited rather low concentrations of tocopherols. Further work on individual and seasonal variation in the content of tocopherols was therefore limited to four of the easily available Fucaceae.

Knowledge of the individual variation in the plant material is a necessary prerequisite for the safe estimation of average composition and possible seasonal or other variations of any component of the material in question. The individual variation in the content of dry matter, ash, and alginic acid has been determined for a number of Norwegian algae.[2] However, one cannot apply the standard deviations established for these components to the tocopherol group, since the latter comprise highly active metabolites compared with the rather inert main components. The individual variation in tocopherol content therefore had to be established separately. One might also expect diurnal variations in the tocopherol content since Booth[3] has reported that the tocopherol content of leaves harvested in bright light was lower than that of leaves growing in dull illumination. In the present paper the results of an attempt to determine the variations in tocopherol content of four common littoral seaweeds are reported.

Experimental

The algal samples were collected at Flakk in the Trondheimsfjord. For the determination of the individual variation in the material 10 individual *Ascophyllum nodosum* plants were taken simultaneously from a homogeneous population on 22 August, 1968. The material was brought to the laboratory and analysed immediately for dry matter and α-tocopherol content.

To determine the seasonal variation, samples of the algae *Ascophyllum nodosum*, *Pelvetia canaliculata*, *Fucus serratus* and *Fucus vesiculosus* were collected each month. Each sample was taken from the same homogeneous population and consisted of at least 20 individual plants. The material was brought to the laboratory and analysed immediately.

The diurnal changes in individual tocopherols were determined with samples taken every third hour throughout a 24 h period from a homogeneous stand of *A. nodosum*. The experiment was carried out on 2 September, 1968. Each sample consisted of the apex and the upper three internodes of 25 plants.

The distribution of individual tocopherols in two *A. nodosum* plants was also determined by analysing separately tissue samples of identical age. Unripe receptacles were removed from the thallus, and the cleaned plant was cut as shown in Fig. 1.

In two instances the ripe fruiting receptacles were separated from the thallus of *A. nodosum* plants and analysed separately.

The determination of the individual tocopherols was carried out as described earlier.[1] The algal samples were usually passed through a Wiley laboratory mill fitted with a 2 mm perforated screen, and a suitable aliquot was quartered out for analysis. The handling of the material and all analytical operations were carried out in subdued light.

Results

The individual variations in dry matter and α-tocopherol content of ten *A. nodosum* plants are shown in Table I.

Only α-tocopherol was determined in order to be able to finish the whole series of analysis in one day. The average content was found to be 91·8 mg α-tocopherol/kg of dry weight, and the standard error (σ_m) was 5·6 mg/kg or 6·1%, provided that the small number of determinations carried out gave a correct estimate of the total variance in the material and method.

The contents of individual tocopherols in the different parts of two *A. nodosum* plants are shown in Figs 1 and 2.

In the second case samples 1 and 3 were analysed immediately upon arrival at the laboratory while the rest of the samples were kept at −20° for one day prior to analysis.

*Part I: Preceding paper

TABLE I

Individual variation in dry matter and α-tocopherol content of *Ascophyllum nodosum*, Flakk, 22 August, 1968

Plant No.	Dry matter, %	α-Tocopherol, mg/kg dry matter
1	30·9	111·1
2	33·7	91·2
3	31·8	68·3
4	35·3	113·8
5	35·4	116·3
6	32·0	99·2
7	34·6	75·8
8	31·2	78·6
9	33·6	91·9
10	30·6	71·9

M = 91·8 mg/kg; σ_m = 5·6 mg/kg

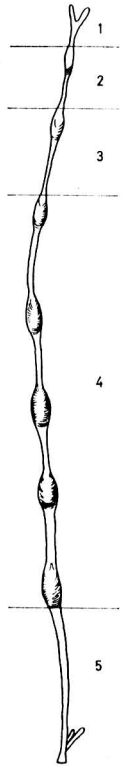

FIG. 1. *Content of dry matter and α-tocopherol in different parts of* Ascophyllum nodosum, *Flakk, 20 August, 1968*

Sample	Dry matter, %	α-Tocopherol, mg/kg dry matter
1	25·6	68·3
2	32·2	70·0
3	35·5	87·4
4	—	—
5	34·7	195

The data from the investigation of the diurnal changes in composition within an *A. nodosum* population have been collected in Table II.

The analytical figures for the tocopherol contents of *A. nodosum*, *F. serratus*, *F. vesiculosus* and *P. canaliculata* covering a two-year period are given in Figs 3, 4, 5 and 6, and Table III shows the tocopherol content of ripe fruiting receptacles of *A. nodosum*.

Discussion

The individual variation in the α-tocopherol content of plants belonging to a seemingly homogeneous population of *A. nodosum* was found to be considerably higher (σ_m = 6·1%) than for the dry matter content (σ_m = 1–3%) as previously established by Baardseth & Haug.[2] It was however, within the range of variation in ash content found for *A. nodosum* plants by Baardseth.[4] Since the seasonal variations were investigated for samples containing more than 20 individual plants, the standard deviation of the corresponding means will be reduced by the square root of two. This applies to

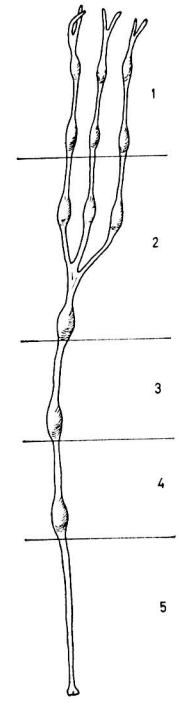

FIG. 2. *Content of dry matter and individual tocopherols in different parts of* Ascophyllum nodosum, *Flakk, 30 August, 1968*

Sample	Dry matter, %	Tocopherol, mg/kg dry matter		
		α	γ	δ
1	37·1	87	94	310
2	38·0	150	150	456
3	34·2	210	160	430
4	34·2	190	133	319
5	35·9	140	74	285

the error arising from the individual variations. To this must be added variations caused by possible differences in age-distribution between samples. This difference might be considerable in the case of *A. nodosum* since the apex was found to contain less than half the quantity of the α-tocopherol present in older parts of the plant. However, the samples usually consisted of whole plants of similar age, and the variations in age-composition were kept as small as possible.

It should be noted that the different tocopherols varied in concentration between different parts of the plant. Rapidly growing tips were poorer in all tocopherols than were the oldest internodes, which had passed their optimum growth period. Booth & Hobson-Frohock[5] have reported that the α-tocopherol content is low in rapidly growing leaves, and that the concentration increases towards shedding. It was observed by the present author that the tissue of the apex appeared light yellow to greyish in colour while that of the lower internodes was dark green. Quantitative paper chromatography showed that the concentration of chloroplast pigments of the older tissue could be twice that of the young cells.

The investigation of the diurnal variation in tocopherol content of *A. nodosum* indicated a number of important possibilities and interrelationships. The α- and δ-tocopherols seemed to vary inversely. While the concentration of δ-tocopherol increased toward the end of the desiccation and in the first part of the submerged period, the quantity of α-tocopherol decreased during the emerged period. The effects observed are believed to be mainly connected with submersion/emersion phenomena since the dark period was quite short (4–5 hours). It seems that further investigations along these lines might contribute considerably to knowledge of the biochemistry of the tocopherols in these plants. Of importance in connexion with the present work is the fact that the diurnal

TABLE II

Changes in tocopherol content of *Ascophyllum nodosum* throughout the day

No.	Collection time	Condition	Dry matter, %	Tocopherol, mg/kg dry matter		
				α	γ	δ
1	09.00	submerged	27·3	84	62	247
2	12.00	dry	29·3	72	69	242
3	15.00	dry	32·6	80	66	258
4	18.00	submerged	29·8	53	55	291
5	21.00	submerged	32·3	93	67	269
6	24.00	dry	27·9*	93	70	236
7	06.00	submerged	30·8*	75	63	210
8	09.00	submerged	27·7	75	72	237

* Samples 6 and 7 were stored at −20°C for 8 and 4 hours respectively prior to analysis

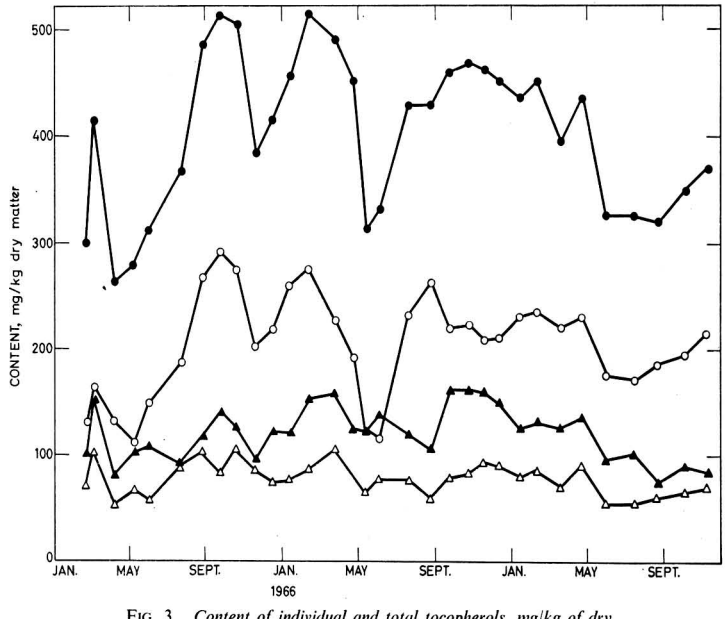

FIG. 3. *Content of individual and total tocopherols, mg/kg of dry matter for* Ascophyllum nodosum

● Total tocopherols; ○ δ-Tocopherol;
▲ α-Tocopherol; △ γ-Tocopherol

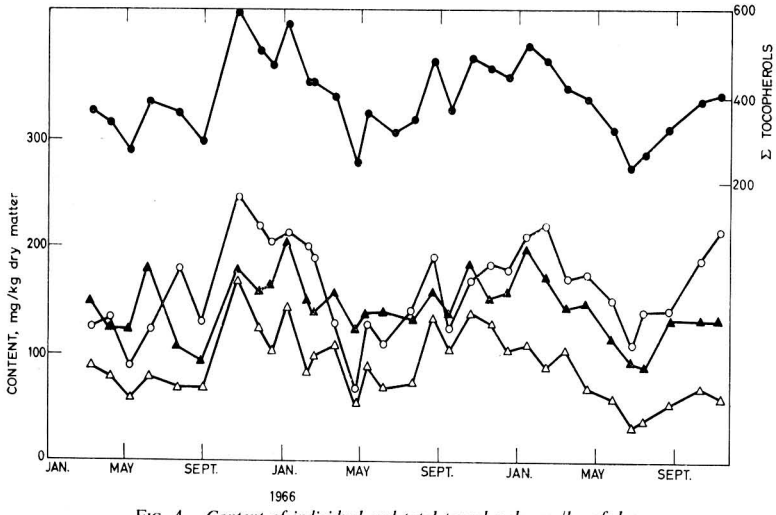

FIG. 4. *Content of individual and total tocopherols, mg/kg of dry matter for* Fucus serratus
Symbols as in Fig. 3

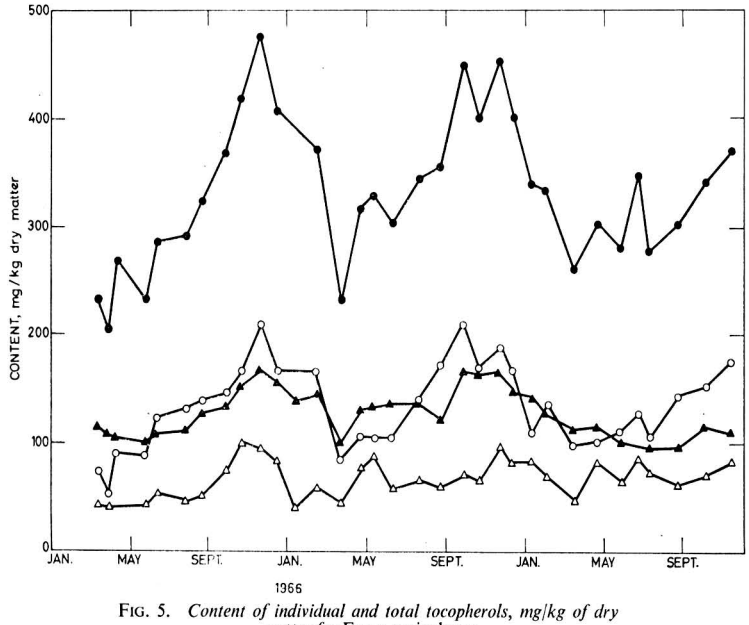

FIG. 5. *Content of individual and total tocopherols, mg/kg of dry matter for* Fucus vesiculosus
Symbols as in Fig. 3

changes in tocopherol content may give fluctuations of the order of 10% around the average value of the season.

The marked variations shown in Figs 3–6 for the content of δ-tocopherol and total tocopherols are too pronounced to be accidental. It is therefore concluded that all the four seaweeds investigated exhibited considerable seasonal variation in their content of tocopherols. Samples collected in winter contained approximately twice the concentrations found in summer samples. These changes were mainly caused by the variation in the δ-tocopherol content, although all three tocopherols varied in a somewhat parallel way in the two *Fucus* species.

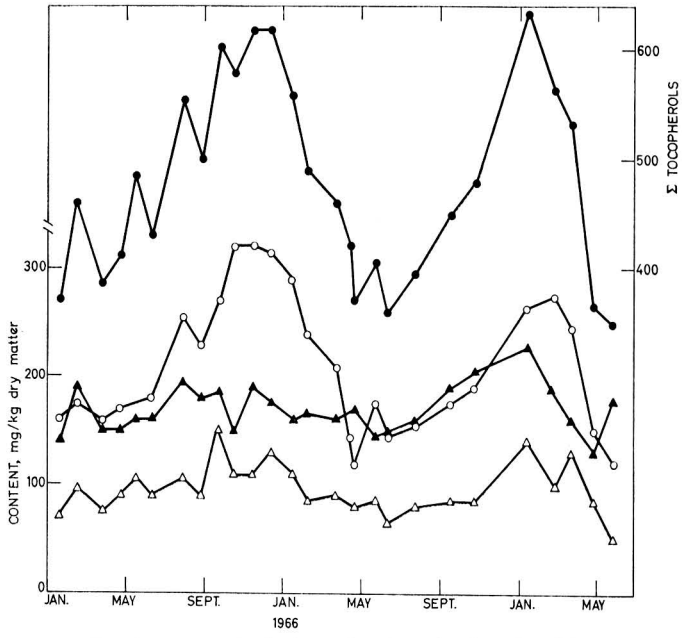

FIG. 6. *Content of individual and total tocopherols, mg/kg of dry matter for* Pelvetia canaliculata
Symbols as in Fig. 3

TABLE III

Tocopherol content of ripe fruiting receptacles of *Ascophyllum nodosum*, mg/kg of dry matter

Date of harvest	α-Tocopherol	γ-Tocopherol	δ-Tocopherol
7 May, 1965	60	30	45
12 May, 1966	100	28	30

There were indications of seasonal variations in the data given by Brown[6] for the content of tocopherols in a number of marine algae. However, as was pointed out by Brown himself, the differences between autumn and winter samples may have been caused by the relatively prolonged storage of the winter samples compared with the material collected in autumn. Since it was found in the present investigation that samples collected in September and January differed very little in the concentration of tocopherols, it seems that the storage must have been the main cause of the differences observed by Brown. Effects of storage on the tocopherol content of seaweed meal will be dealt with in the following paper[7] of this series.

A closer inspection of Figs 3–6 reveals some significant differences between the algae investigated. The seasonal variations were most pronounced for *P. canaliculata* and this alga showed the highest content of total tocopherols. It was also on an average richest in the alpha homologue. *F. serratus* showed the highest proportion of α-tocopherol to total tocopherols. For both of the two *Fucus* species investigated the quantities of α- and δ-tocopherols were nearly equal, while in the case of *P. canaliculata* and *A. nodosum* the δ-homologue was the dominating component. *A. nodosum* was characterised by a low proportion of α- to non-α-tocopherols.

Analysis of ripe fruiting receptacles (Table III) revealed that the content of α-tocopherol of this part of the plant remained normal while the concentration of δ-tocopherol was about one-fifth of that of the thallus. Several authors have shown that α-tocopherol is mainly located in the chloroplasts,[8,9] whereas tocopherols other than the α-homologue are not so closely associated with chlorophyll. On a dry matter basis the ripe receptacles are dominated by cortical tissue, i.e. cells with a high proportion of chromatophores, even in comparison with tissue from the oldest internodes of the plant. It could be surmised that the concentration of δ-tocopherol is smaller in the less pigmented receptacles.

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THEORETICAL TRIGLYCERIDE CONTENT OF VEGETABLE OILS BY A RADIOCHEMICAL TECHNIQUE—EFFECT OF SEDIMENT

By C. A. MARCOPOULOS and K. A. MANOLKIDIS

The refining loss for a variety of olive, maize and cottonseed oils as determined in the laboratory by a chromatographic method was checked by a radiochemical procedure. By labelling oil samples with ^{14}C-tripalmitate and applying the principle of isotope dilution analysis, the absolute content of neutral oil and the theoretical triglyceride content were determined.

In order to find out the effect of the sediment in an oil on the deviation of the chromatographic refining loss from the theoretical triglyceride content, various oil samples with high amounts of sediment were compared with normal samples with a low or moderate percentage of sediment.

Relations are proposed for the calculation of the theoretical triglyceride content from the one determined by the chromatographic method.

Introduction

The refinery yield of neutral oil has been determined by various methods.[1–3] The actual percentage of neutral triglycerides is usually determined by one of three standard methods: the 'acetone-insoluble' method,[4–9] the Wesson method[10–12] and the chromatographic method.[13–21]

Since the actual results of these methods differ, attempts have been made to establish a correlation between them. Purdum & Werber[8] determined the refining loss of many samples of cottonseed and soyabean oil by the Wesson and acetone-insoluble methods and found relationships between them and the 'cup test'.[3] Marcopoulos[22] examining the Wesson, acetone-insoluble and chromatographic methods for pistachio seed oil, found relations between them and between the actual percentage of neutral triglycerides according to the chromatographic method and the free fatty acid (*FFA*) content.

Some uncertainty exists about the precision of these methods, and the absolute neutral oil content of a vegetable oil is not easy to determine with accuracy. The difficulty in any standard method is in isolation of the whole amount of the neutral triglycerides in the oil, and a technique by which the absolute neutral triglyceride content of a mixture in a substance may be determined without its complete isolation is desirable.

In a previous paper,[23] the control of the chromatographic method by a radiochemical technique, the determination of the absolute neutral oil content in a variety of Greek cottonseed oils and the comparison of the chromatographic refining loss with the radiochemical technique were reported. A relation giving the theoretical triglyceride content from the chromatographic loss was proposed.

Since it was found that neutral triglycerides were not retained on the alumina column when in a chemically pure form, it seems that the percentage of an oil retained and analysed by the chromatographic method depends on other substances found in the oil.

It is important to know whether the deviation of the chromatographic refining loss is due to the *FFA* content or to impurities. This work concerns control of the chromatographic method by radiochemical technique for a variety of Greek vegetable oils and examination of the effect of the sediment.

Experimental

Materials and Apparatus

Chromatographic columns were constructed according to the official method of A.O.C.S.[21] Alumina (grade F 20, mesh 80–200 from the Aluminium Company of America, Chicago, Illinois) was used. Before use it was treated according to the official method of A.O.C.S.[21] A mixture of ether–methanol (97·5 : 2·5% by vol.) was used as solvent.

Glyceryl-1-^{14}C-tripalmitate (from Radiochemical Centre, Amersham, Bucks., England) was used as labelled triglyceride. ^{14}C-tripalmitate was used as the carrier-free solution (solution A). This was prepared by dissolving 0·02 g glyceryl-1-^{14}C-tripalmitate in 200 ml ether–methanol solution (total activity 1 μCi).

Counting techniques

The liquid-scintillation counting technique which has already been reported, was applied here as the more convenient method for detecting the low activities of β-radioactive ^{14}C. As liquid scintillator, a solution of 4 g 2,5-biphenyloxasol and 0·2 g 1,4-bi-5(2-phenyl)oxazolyl benzene, in 1 ml of toluene was used (solution Sc).

Active samples were measured in small glass tubes (2 cm dia. × 5 cm height), 15 min after restoration in the counter's refrigerator (Packard Tricarb Liquid Scintillation Counting System, Model 314 EX).

Measurements took place under conditions of high voltage–1050 V, amplitude 1000, window 100–700 V. The amount of radioactive tripalmitate used was calculated in advance each time, in order to give a count-rate of the order of 10,000 counts/3 min in the active samples measured. Under these conditions, the efficiency of the counting system was found to be 66%. The measurements were checked by measuring the same sample repeatedly; the relative standard deviation of the values obtained was found to be less than 1%.

Procedure

To determine the absolute content of neutral oil the principle of the 'isotope dilution analysis' was applied. Separation of the neutral triglycerides was carried out by the chromatographic method.

A known amount of oil containing the unknown weight of neutral triglycerides W_o, was mixed with the known weight W_1 of pure labelled triglyceride, total radioactivity R. The specific activity S_1 of W_1 may be found from the relation:

$$S_1 = \frac{R}{W_1} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

The total amount of triglycerides in the mixture is now $W_o + W_1$ with the same total activity R but with the specific activity S_2 given by the relation:

$$S_2 = \frac{R}{W_o + W_1} = \frac{R'}{p} \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

where R' is the total activity of a fraction p of the quantity $W_o + W_1$.

For the determination of S_2 it was not necessary to separate the total amount of $W_o + W_1$ but only the fraction p, no matter what percentage of $W_o + W_1$ had been retained on the column. By comparing Equations (1) and (2), the quantity W_o may be calculated without absolute separation:

$$W_o = \frac{W_1 (S_1 - S_2)}{S_2} \quad \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

In the chromatographic method for the determination of the absolute content of neutral triglyceride, W_o should be thoroughly separated; but if a fraction of it is retained on the column besides *FFA* and impurities, the result is a higher chromatographic loss. By applying the isotope dilution analysis and assuming that ^{14}C-tripalmitate is entirely dispersed through the neutral triglycerides of the oil, W_o may be calculated no matter what fraction of it was retained.

To take always the same amount of W_1, 0·02 g of ^{14}C-tripalmitate were dissolved in a 200 ml volumetric flask with ether–methanol solution and the flask was made up to volume (solution A). 5 ml (containing 0·0005 g ^{14}C-tripalmitate) were taken each time.

A known amount of oil was diluted in a small Erlenmeyer flask with 15 ml of solvent (10 ml ether–methanol solution and 5 ml solution A). After the solution had been thoroughly homogenised, it was transferred to the alumina column. The rate of passage, the washing and the collection of the eluate from the chromatographic column, were followed as described previously.[21] A slight modification was made in order that a weight p representative of the whole amount of the eluate was always taken, thus avoiding errors from the difference in percentage of the eluate from the first to the last fraction. Instead of collecting the percolate with the washings in a Soxhlet flask, they were collected in a 200 ml volumetric flask which was then filled with ether–methanol solution. After the solution had been thoroughly homogenised, 20 ml were taken and evaporated in a glass tube. After drying, cooling and weighing (wt. p), 5 ml solution Sc and 5 ml ether–methanol solution were added, the content was dissolved and the activity R' was measured. The specific activity S_2 was calculated.

Since preliminary experiments showed that the count-rate of active samples varied with the density and the colour of the solution, S_1 should be calculated under the same conditions of determination as S_2. Therefore for each sample a blank was determined as follows.

The same amount of oil was dissolved with 15 ml ether–methanol solution (no radioactive addition) and transferred to the column. The percolate and the washes were collected in a 200 ml volumetric flask, 5 ml solution A were added and the flask was made up to volume with ether–methanol solution. 20 ml were taken treated as above and its radioactivity was counted.

From Equation (3), W_o (the absolute neutral oil content) was calculated.

Results and Discussion

The refining loss and the neutral oil content were determined by both the radiochemical procedure and the standard chromatographic one for a variety of Greek cottonseed, maize and olive oils.

For each sample the determination was carried out in triplicate and mean values were calculated. In all cases, the chromatographic refining loss was found to be a little greater than the one calculated by the radiochemical procedure. It is assumed that besides *FFA* and impurities, a small percentage of neutral glycerides was also retained in the chromatographic method. If N_r is the percentage of neutral oil retained on the column, the percentage of neutral oil obtained by the chromatographic method N_c is given by:

$$N_c = 100 - (F + I + N_r) \quad \ldots\ldots\ldots\ldots (4)$$

where F and I are the percentages of *FFA* and impurities respectively contained in the oil.

On the other hand N_r is given by:

$$N_r = C - R \quad \ldots\ldots\ldots\ldots\ldots\ldots (5)$$

where C is the chromatographic loss given by:

$$C = F + I + N_r \quad \ldots\ldots\ldots\ldots\ldots\ldots (6)$$

and R the theoretical loss given by:

$$R = F + I \quad \ldots\ldots\ldots\ldots\ldots\ldots (7)$$

In order to examine whether N_r was connected with F and I, two groups of olive oils were taken. The samples of the first group (Table I) were collected from areas giving poor oils. The majority of them had a high *FFA* content and appeared to have a high amount of sediment. The second group (Table II) consisted of a number of normal samples with a moderate content of *FFA* and sediment. In both cases, a deviation of the chromatographic loss from the theoretical one has been observed. However, this deviation is higher for the poorer oils.

In order to find out whether this deviation is due mainly to the *FFA* content or to the impurities, samples for both maize and cottonseed oils were collected and grouped as follows: Group I consisted of normal samples with usual range in F and I content. Group II consisted of the poorest samples that it was possible to collect. From these samples the sediment was partly removed, and *FFA* were added in chemically pure form. The mean value of the total amount of impurities (and unsaponifiable matter) given by the difference between theoretical loss and F (Equation 7), has been found to be higher in Group II than in Group I but the mean percentage of the impurities in relation to the *FFA* content, i.e. the ratio $\frac{100\,I}{F+I}$ which equals the ratio $100\,\frac{(R-F)}{R}$ was higher in Group I than in Group II.

For all samples the content of neutral oil was calculated by both the chromatographic and the radiochemical procedures in triplicate. The mean values of the chromatographic (C,%) and the radiochemical (R,%) refining loss are given in Tables III–VI.

For each group, the mean values of the chromatographic refining loss were plotted against mean values of the radiochemical one. All plots gave straight lines, the slopes and the intercepts of which were calculated by the method of least squares.

In all cases, the correlation coefficients were a good criterion of linearity. On the other hand the standard

TABLE I

Refining loss of Greek olive oils

Group I: poor samples

Sample No.	*FFA*, %	Refining loss		100 (*R*−*F*)	100 (*C*−*R*)
		R, %	*C*, %	*R*	*R*
1	5·6	6·8 ± 0·14	7·3 ± 0·30	17·6	7·4
2	7·6	7·7 ± 0·35	8·4 ± 0·10	1·3	9·1
3	9·3	9·9 ± 1·17	11·0 ± 0·17	6·6	11·1
4	10·4	11·3 ± 1·00	12·9 ± 0·26	8·0	14·2
5	14·6	15·2 ± 0·10	16·7 ± 0·16	4·0	9·9
6	15·1	16·8 ± 0·02	18·3 ± 0·39	10·1	8·9
7	18·5	19·0 ± 0·14	20·9 ± 0·20	2·6	10·0
8	22·4	23·4 ± 0·10	26·0 ± 0·07	4·2	11·1
9	26·3	27·9 ± 0·10	30·4 ± 0·25	5·7	9·0
10	30·8	32·2 ± 0·06	35·2 ± 0·25	4·4	9·3

TABLE II

Refining loss of Greek olive oils

Group II: moderate samples

Sample No.	*FFA*, %	Refining loss		100 (*R*−*F*)	100 (*C*−*R*)
		R, %	*C*, %	*R*	*R*
1	0·4	0·4 ± 0·17	0·4 ± 0·10	0	0
2	4·8	5·1 ± 0·50	6·3 ± 0·20	6·0	23·5
3	6·2	6·8 ± 0·30	6·9 ± 0·04	8·8	1·5
4	6·5	7·3 ± 0·50	7·4 ± 0·60	10·9	1·4
5	7·9	8·2 ± 0·40	8·4 ± 0·04	3·6	2·4
6	7·9	8·6 ± 0·30	9·2 ± 0·16	8·1	7·0
7	8·4	9·0 ± 0·04	9·2 ± 0·32	6·6	2·2
8	12·1	13·5 ± 0·33	14·5 ± 0·11	10·4	7·4
9	12·4	13·9 ± 0·15	14·5 ± 0·04	10·7	3·9
10	19·8	20·9 ± 0·30	22·4 ± 0·70	5·3	7·2

TABLE III

Refining loss of Greek maize oils

Group I: high fraction of sediment in relation to *FFA*

Sample No.	*FFA*, %	Refining loss		100 (*R*−*F*)	100 (*C*−*R*)
		R, %	*C*, %	*R*	*R*
1	0·2	0·2 ± 0·06	0·2 ± 0·09	0	0
2	2·8	3·8 ± 0·04	4·2 ± 0·09	26·3	10·5
3	5·1	6·2 ± 0·40	6·9 ± 0·21	17·7	11·2
4	8·2	9·4 ± 0·09	9·9 ± 0·29	12·8	5·3
5	10·7	11·5 ± 0·06	12·6 ± 0·11	7·0	9·6
6	13·0	13·6 ± 0·90	14·6 ± 0·10	4·4	13·6
7	13·0	13·5 ± 0·13	15·7 ± 0·17	3·7	16·2
8	13·5	14·5 ± 0·01	15·7 ± 0·04	6·9	8·2
9	15·0	15·5 ± 0·30	16·3 ± 0·29	3·2	5·1
10	16·9	17·5 ± 0·04	19·0 ± 0·09	3·4	8·8

TABLE IV

Refining loss of Greek maize oils

Group II: low fraction of sediment in relation to *FFA*

Sample No.	*FFA*, %	Refining loss		100 (*R*−*F*)	100 (*C*−*R*)
		R, %	*C*, %	*R*	*R*
1	19·5	20·5 ± 1·00	21·4 ± 0·04	4·9	4·3
2	24·8	24·9 ± 1·80	26·1 ± 0·11	0·4	4·8
3	25·2	25·4 ± 0·55	26·2 ± 0·03	0·8	3·1
4	26·9	27·8 ± 0·17	29·1 ± 0·04	3·2	4·6
5	28·8	29·8 ± 2·20	31·8 ± 0·07	3·3	6·7
6	30·4	30·6 ± 0·46	33·1 ± 0·11	0·7	8·1
7	31·5	32·4 ± 0·64	33·9 ± 0·20	2·8	4·6
8	32·7	34·0 ± 0·29	34·9 ± 0·17	3·9	2·3
9	35·2	38·6 ± 0·30	40·0 ± 0·04	6·2	3·6
10	38·1	40·3 ± 0·58	42·7 ± 0·40	5·5	5·9

TABLE V

Refining loss of Greek cottonseed oils

Group I: high fraction of sediment in relation to *FFA*

Sample No.	*FFA*, %	Refining loss		100 (*C*−*F*)	100 (*C*−*R*)
		R, %	*C*, %	*R*	*R*
1	0·2	0·2 ± 0·04	0·2 ± 0·04	0	0
2	5·0	5·2 ± 0·30	5·8 ± 0·12	3·8	11·5
3	8·8	9·1 ± 0·10	9·2 ± 0·06	3·3	1·1
4	10·1	10·5 ± 0·14	11·8 ± 0·06	3·8	12·4
5	10·9	12·7 ± 0·26	13·8 ± 0·06	14·2	8·6
6	12·4	13·5 ± 0·04	14·8 ± 0·14	8·1	9·6
7	12·4	13·8 ± 0·15	15·0 ± 0·14	10·1	8·7
8	14·4	15·6 ± 0·24	16·6 ± 0·32	7·6	6·4
9	16·2	17·5 ± 0·32	19·1 ± 0·10	7·4	9·1
10	20·3	20·7 ± 0·17	22·1 ± 0·04	2·0	6·8

TABLE VI

Refining loss of Greek cottonseed oils

Group II: low fraction of sediment in relation to *FFA*

Sample No.	*FFA*, %	Refining loss		100 (*R*−*F*)	100 (*C*−*R*)
		R, %	*C*, %	*R*	*R*
1	24·0	24·1 ± 0·27	25·4 ± 0·10	0·4	5·4
2	24·2	24·5 ± 0·63	26·2 ± 0·17	1·2	6·9
3	26·5	29·2 ± 0·04	30·5 ± 0·07	9·2	4·8
4	27·3	27·8 ± 0·11	28·7 ± 0·07	1·8	3·2
5	27·8	29·1 ± 0·55	30·8 ± 0·04	4·4	5·8
6	27·9	29·0 ± 0·13	30·6 ± 0·04	3·7	5·5
7	28·2	28·3 ± 0·60	30·1 ± 0·21	0·4	6·3
8	28·7	32·3 ± 0·75	33·9 ± 0·10	11·1	5·0
9	32·7	35·1 ± 0·79	37·4 ± 0·17	6·8	6·6

TABLE VII

Parameters of the relations between chromatographic (*C*) and theoretical (*R*) refining loss

General relation: $(R\%) = \frac{(C\%)-a}{b}$

Oil group	Slope (*b*)	Standard Deviation (*Sb*)	Intercept (*a*)	Standard Deviation (*Sa*)	Correlation coefficient (r)
Olive oils I	1·091	±0·009	0·135	±0·16	0·999
Olive oils II	1·063	±0·025	−0·040	±0·24	0·999
Maize oils I	1·083	±0·026	0·065	±0·23	0·998
Maize oils II	1·051	±0·030	−0·058	±0·86	0·998
Cottonseed oils I	1·076	±0·018	0·060	±0·22	0·999
Cottonseed oils II	1·063	±0·035	−0·230	±1·00	0·998

deviation of *a* was greater than *a*, raising doubts about the existence of intercepts. This was expected, however, since previous experiments,[23] in which neutral triglycerides were extracted thoroughly from the column showed that, in the case where the theoretical refining loss equals zero, no chromatographic refining loss could be observed. Therefore, *a* should be ignored in all cases and direct relations between chromatographically determined and theoretical refining loss should be established as in Table VII.

Tables III–VI also show that the higher the percentage of impurities in relation with the total theoretical loss ($F + I$), the greater is the deviation of the chromatographically determined loss from the theoretical loss. A similar relation is not shown by Tables I and II. This may be due to the variation of *FFA* and impurities in the natural samples examined. However, even in the latter case, the poorer oils gave a higher mean deviation. It may be concluded, as a first approximation, that in samples of natural oil, a difference between theoretical and chromatographic loss exists, owing to neutral oil being retained on the column besides *FFA* and impurities. This difference possibly increases by increasing the ratio $I/F + I$, i.e., by increasing the amount of impurities in relation to the *FFA* content.

Owing to the various physical and chemical factors which effect this phenomenon, exact relations are difficult to find. For the Greek vegetable oils it may be concluded that the amounts of neutral triglycerides retained on the column have a mean value of 5% of the total theoretical refining loss ($F + I$), for common oil samples, reaching the mean value of 9% for very poor oil samples. However, it must be noted that the standard deviation of this mean is high, owing to the variability of the samples, and in some cases the deviation could reach 20% of the total theoretical refining loss.

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SEMI-AUTOMATIC METHODS OF EVALUATING THE QUALITY OF TEA INFUSIONS

By C. B. CASSON and A. JEAN SHENTON

The Roberts & Smith procedure for determining polyphenols and colour in tea infusions has been adapted to semi-automatic operation at the rate of 40 samples per hour. Several modifications have been made, especially in the theaflavin determination. These have not affected reproducibility, and results by the two methods have shown adequate correlation. Examples are given of the application of the method to the constituents of a tea blend; the optical density values for the blend are predictable from the values for the individual teas. Individual garden teas show wide variations in polyphenol content; these variations have not yet been correlated with organoleptic properties.

Introduction

The traditional methods of evaluating and classifying leaf teas are by the appearance of the leaf and by the odour, taste and appearance of the infusion. This work makes great demands on the skill and experience of highly trained tasters, and in principle, it is possible to supplement at least some of their work by instrumental measurement. One such measurement might reflect the organoleptic properties of the polyphenols. A method of evaluating polyphenols in tea infusions has been proposed by Roberts & Smith.[1] This method involves manual solvent extraction of the infusion followed by a series of spectrophotometric measurements on the fractions obtained. As it stands the method is too time-consuming for application to the quality control of tea, and semi-automatic modifications have therefore been developed that are capable of dealing with tea infusions at the rate of forty samples per hour. For this purpose the Technicon AutoAnalyzer has been used.

Experimental

A number of modifications were introduced into the Roberts & Smith method in adapting it to the AutoAnalyzer; the methods as finally worked out are given below. In describing the AutoAnalyzer procedure a general knowledge of the principles and practice of using this equipment is assumed.[2] It has been found necessary in this work to have all the reagents flowing through the manifold and to have water flowing through the sample line, before the base line is set on the chart.

Preparation of the tea infusions

Apparatus

The apparatus is shown in Fig. 1. The pan of a bench-type spring balance, graduated 0–500 g in 2 g intervals and fitted with a rotatable scale so that it could be used for taring, was replaced by a suitable stirrup to hold a half-pint vacuum flask. A bottle shaker, oscillating in a horizontal plane at about 100 cycles/min with an amplitude of approximately 7 cm was fitted with a box into which six vacuum flasks could be packed with their longitudinal axes in the direction of oscillation.

Procedure

4·5 g of the sample of leaf tea was introduced into a warmed vacuum flask with the aid of a funnel. The flask and funnel assembly were placed in the stirrup of the balance, which was tared, and 187 g of boiling distilled water was run in from the boiler. The flask was securely stoppered and was shaken for 8 min in the shaker described. The infusion was strained through muslin at the end of the shaking period and cooled immediately in a stoppered vessel.

Evaluation of total colour

An AutoAnalyzer consisting of a sample plate, proportioning pump, colorimeter with 460 nm filters, recorder, and manifold were set up as shown in Fig. 2. Having assembled the relevant manifold in Fig. 2, the sample plate was set at 40

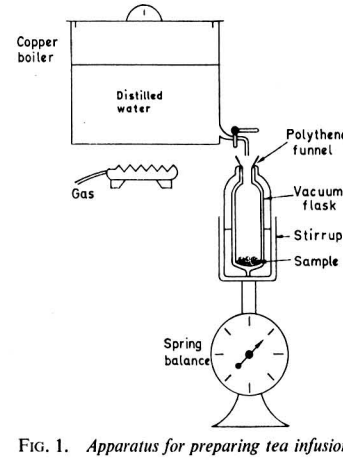

FIG. 1. *Apparatus for preparing tea infusions*

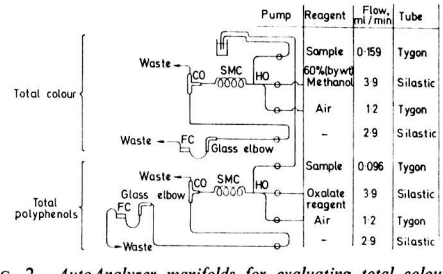

FIG. 2. *AutoAnalyzer manifolds for evaluating total colour and total polyphenols in tea infusions*

SMC, single mixing coil; HO, CO, Technicon codes for glassware; FC, flow cell (460 nm for total colour and 380 nm for total polyphenols)
Oxalate reagent: (10 g oxalic acid, 750 ml methanol (99–100%) and 400 ml water

samples/h and was loaded with the sample infusions in accordance with the general procedure for AutoAnalyzer determinations.[2]

Evaluation of total polyphenols

The manifold for total polyphenols (Fig. 2) was assembled and using 380 nm filters the evaluation was carried out in accordance with AutoAnalyzer general instructions at a sampling rate of 40 samples/h. The oxalate reagent used contained 10 g oxalic acid, 400 ml distilled water and 750 ml 99–100% methanol. If a two-pen recorder is available this manifold and the one for total colour may be run concurrently.

Evaluation of theaflavin

Apparatus and reagents

The AutoAnalyzer modules used for total polyphenols, were fitted with the manifold shown in Fig. 3. This included three displacement bottles, of all-glass construction and conveniently of 1 litre capacity.

Tris buffer: 60·7 g tris (hydroxymethyl) methylamine was dissolved in 550 ml 0·5 N hydrochloric acid and distilled water to 2500 ml was added. The solution was adjusted to pH 8·0 by the addition of 0·5 N hydrochloric acid and mixing, 2·5 ml Teepol was added, and the reagent was re-mixed.

Procedure

The manifold was assembled as shown in Fig. 3 and a sampling rate of 40 samples/h was arranged. At the start the displacement bottle 1 was full of methylisobutyl ketone and the others were full of water. Water pumped into bottle 1 displaced the ketone into the manifold and bottles 2 and 3 aspirated the aqueous phase and some ketone out of the manifold. The pump tube sizes given should ensure that only ketone flows from the upper outlet of the separator B2; slight adjustment may be necessary in some cases to ensure that no aqueous phase is entrained in the solvent phase.

Standards

Large samples of a number of teas of different polyphenol contents were assembled and their total colour, total polyphenols, and theaflavin content were determined on each by the manual methods given below.

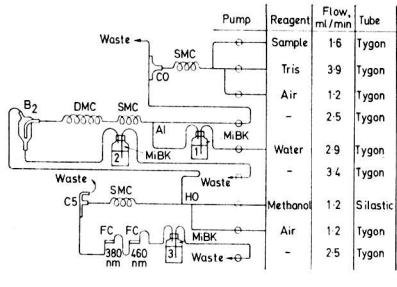

FIG. 3. *AutoAnalyzer manifold for evaluating theaflavin in tea infusions*

SMC, single mixing coil; DMC, double mixing coil; 1,2,3, displacement bottles; HO, CO, Al, B2, C5, Technicon codes for glassware; FC, flow cell, MiBK, methyl-isobutyl ketone

Trisbuffer: 60·7 g tris (hydroxymethyl) methylamine, 550 ml 0·5N-HCl, made up to 2·5 l with water. Adjust to pH 8 by addition of 0·5N-HCl, mix, add 2·5 ml of Treepol and remix

Total colour

2 ml of infusion was pipetted into 30 ml of 60% methanol. After mixing, the optical density was measured at 460 nm in a 1 cm cell in a suitable spectrophotometer, using 60% methanol in the reference cell.

Total polyphenols

2 ml of infusion were pipetted into a 50 ml graduated flask and diluted to the mark with the specified oxalate reagent. After mixing, the optical density was read in a 1 cm cell at 380 nm using oxalate reagent in the reference cell.

Theaflavin

The theaflavin determination was carried out according to the manual procedure recommended by Roberts & Smith,[1] substituting the specified tris buffer for the sodium bicarbonate solution.

4 or 5 teas covering the range of polyphenol contents required were selected and infusions from these teas were introduced on the AutoAnalyzer at the beginning and end of each run.

Results

The methods described have been applied both to individual garden teas and to blends prepared from them. Optical density peak heights observed in a typical run are presented in Table I.

Discussion

Preparation of the Infusions

Unless extreme care was taken the Roberts & Smith method did not give reproducible infusions. The modified procedure gave good replication of results; replicates on the same leaf tea never differed by more than 0·01 optical density units. 'Creaming down' occurred when the infusions were cooled but provided that they were shaken before the sample cups were loaded this did not affect the results.

The results in Table II were obtained by shaking for various times tea infusions prepared in the apparatus of Fig. 1. They show that the apparent extraction of theaflavin reached a maximum after 6 min shaking, remained almost constant for a further 6 min and then decreased slightly. The cause of the decrease has not yet been investigated. On the basis of these results it was decided to adopt 8 min as the standard shaking time.

Determination of total colour

Roberts & Smith measured total colour as the sum of solvent-extractable colour and residual colour in the aqueous phase. This seemed an unnecessary complication in an automated method; total colour was therefore measured on the original infusion suitably diluted and with methanol added to ensure that the 'cream' was dissolved. Fig. 4 shows the relationship between optical densities by the manual Roberts & Smith method and those obtained by the semi-automatic method on six representative tea blends. E_A and E_B relate to Roberts & Smith solutions A and B, but the optical densities have been calculated back to the original infusions. The curve shows reasonable correlation between the two methods.

TABLE I

Typical optical density values for individual teas and for a blend prepared from them

Tea sample	Origin	Theaflavin at 380 nm	Total polyphenols at 380 nm	Total colour at 460 nm
1	Ceylon	0·240	0·49	0·37
2	Ceylon	0·295	0·50	0·34
3	Travancore	0·215	0·54	0·38
4	Assam	0·225	0·46	0·38
5	Vietnam	0·250	0·49	0·32
6	Ceylon	0·255	0·51	0·33
7	Ceylon	0·315	0·51	0·30
8	Ceylon	0·285	0·50	0·33
9	Ceylon	0·290	0·51	0·35
10	Ceylon	0·295	0·54	0·36
11	Assam	0·225	0·51	0·43
12	Assam	0·350	0·53	0·46
13	Assam	0·415	0·53	0·47
14	Ceylon	0·270	0·55	0·40
15	Ceylon	0·350	0·55	0·42
16	Assam	0·570	0·65	0·62
17	Assam	0·495	0·61	0·59
18	Assam	0·475	0·59	0·54
19	Assam	0·425	0·58	0·54
20	Kenya	0·380	0·57	0·41
Blend		0·335	0·55	0·42

TABLE II

Effect of time of shaking on apparent extraction of theaflavin

Period of shaking, min	Optical density at 380 nm	
	Sample 1	Sample 2
2	0·26	0·28
4	0·28	0·30
6	0·30	0·32
8	0·30	0·32
10	0·30	0·31
12	0·30	0·31
15	0·29	0·30
20	0·28	0·29
30	0·27	0·27

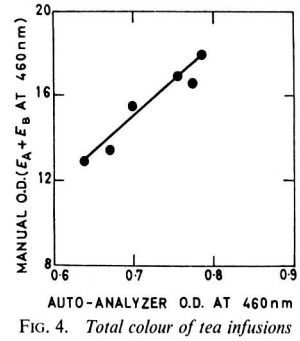

FIG. 4. *Total colour of tea infusions*

Relation between semi-automatic and manual optical densities of infusions from 6 different teas

Determination of total polyphenols

Roberts & Smith measured total polyphenols (thearubigins +theaflavin) as the sum of the optical densities of two solutions, but for the purpose of automatic determination it seemed appropriate to take the optical density of one solution only, namely an acidified infusion to which methanol had been added. The relevant manifold in Fig. 2 was therefore arranged to carry out these operations and to measure the optical density at 380 nm. Fig. 5 shows the correlation between manual results and AutoAnalyzer results on six representative teas. E_A and E_D are the optical densities of Roberts & Smith's solutions A and D, calculated back to the original infusions. The curve shows that the modifications introduced, especially the substitution of one measurement for the two carried out by Roberts & Smith, have not invalidated the results.

Determination of theaflavin

The manual extraction method used for theaflavin is more complicated than those described earlier and its adaptation therefore presented greater difficulties.

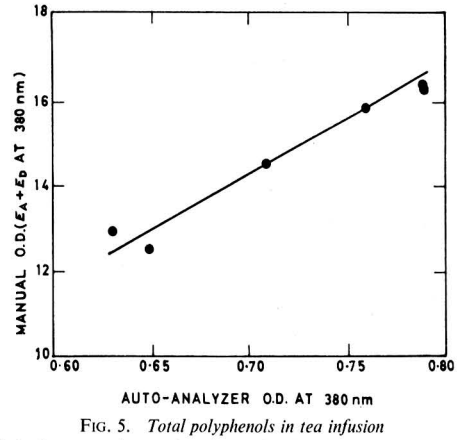

FIG. 5. *Total polyphenols in tea infusion*

Relation between semi-automatic and manual optical densities of infusions from 6 different teas

Modification of the extraction procedure

Roberts & Smith endeavoured to extract all the theaflavin and part of the thearubigins into a suitable solvent and then to wash out the thearubigins with sodium bicarbonate solution. In designing a manifold for theaflavin an attempt was made to carry out the extraction and washing stages in this way, but a reliable system proved difficult to attain. It was decided therefore first to restrict the extractability of thearubigins by adding sodium bicarbonate to the aqueous infusions and then to extract the theaflavin with solvent. Displacement bottles were used to avoid damage to the pump tubes by the solvent. These bottles can be used to pump solvent into or out of a manifold, but they cannot be used directly to meter solvent within a manifold. To effect a phase separation under these conditions the unwanted phase was metered out of the manifold (together with a small proportion of the wanted phase) leaving a suitable proportion of the required phase metered by difference. The difference-metering technique was only feasible in the absence of air segmentation, but following a manifold design of Pentz,[3] air was omitted from the extraction stages and resolution was found to be satisfactory.

Comparison of extraction procedures

AutoAnalyzer peak heights in optical density units were compared with optical densities determined manually on the six infusions used in testing the manifolds described earlier.

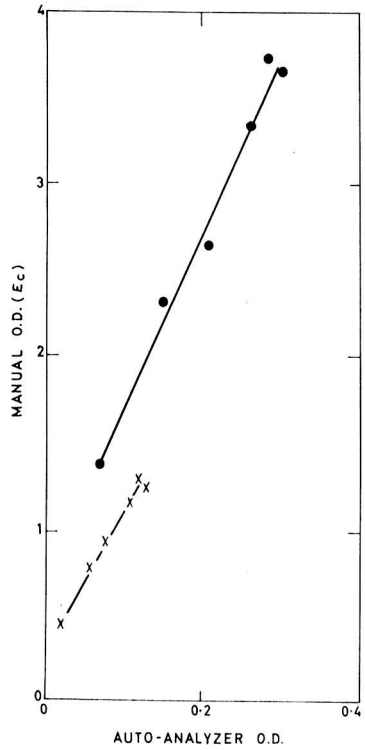

FIG. 6. *Theaflavin in tea infusions*

Relation between semi-automatic and manual optical densities of infusions from 6 different teas
● 380 nm; × 460 nm

The results are shown in Fig. 6. E_C is the optical density of Roberts & Smith's solution C calculated back to the original infusion. The results indicate that peak heights on the chart can be interpreted as theaflavin contents by way of standard teas whose theaflavin content has been determined by the manual method. It is noteworthy that the manual results were obtained by the established procedure and the automatic results by the modified extraction procedure. The results show, therefore, that the modified extraction procedure is practicable.

Control of pH at the extraction stage

Sodium bicarbonate solution when freshly prepared has a pH of 8·0, but on storage in a reagent bottle its pH may rise to 8·7. Furthermore, peaks with old bicarbonate solutions are lower than those with fresh solutions. It was therefore decided to use a buffer solution in place of bicarbonate; a series of buffers was tried and the results are shown in Table III.

Different buffers gave different optical densities even though they were all operating at pH 8·0. Tris and triethanolamine buffers behaved similarly though the optical densities in both cases were slightly higher than those obtained with fresh bicarbonate at the same pH. Phosphate and glycine gave higher optical densities; the cause of this is still under investigation. As tris buffer corresponded reasonably closely to fresh bicarbonate it was chosen to control the pH of the infusions.

Effect of pH on stability of colour

It seemed possible that some degradation of theaflavin might occur in the aqueous phase left to stand at pH 8·0 and that this might affect the reliability of the AutoAnalyzer results. The effect was tested by pumping continuously a buffered tea infusion through a suitably modified manifold to extract theaflavin. No significant change in optical density was observed between 3½ min (the minimum observable period) and 17 min after addition of buffer. Between 17 min and 53 min after raising the pH the optical density fell by 15%. After 53 min the fall in optical density became more rapid. These results suggest that such variation in the holding time at pH 8·0 as may occur in AutoAnalyzer determinations will have a negligible effect on the optical densities. In contrast to these findings, careful manual extractions from an infusion buffered to pH 8·0 did not give reproducible results. It was decided therefore, that when tea infusions were being evaluated manually for calibration purposes, the infusion must first be extracted wtih ketone and the extract must then be washed with buffer solution at pH 8·0, i.e. a procedure similar to that of Roberts & Smith must be followed.

TABLE III

Effect of buffers on theaflavin extraction

Buffer	pH	Optical density at 380 nm			
		Tea 1	Tea 2	Tea 3	Tea 4
Bicarbonate	8·7	0·06	0·14	0·19	0·22
Bicarbonate	8·0	0·12	0·25	0·31	0·33
Tris*	8·0	0·15	0·32	0·38	0·43
Triethanolamine	8·0	0·15	0·34	0·41	0·46
Phosphate	8·0	0·18	0·42	0·49	0·54
Glycine	8·0	0·27	0·56	0·63	0·67

*Tris (hydroxymethyl) methylamine

TABLE IV

Comparison of observed optical density of the blend with the optical density calculated from the data for individual teas

Determination	Wave-length, nm		Optical density of blend				
			Blend A	Blend B	Blend C	Blend D	Blend E
Theaflavin	380	Observed	0·310	0·335	0·370	0·240	0·360
		Calc.	0·315	0·335	0·360	0·245	0·355
Theaflavin	460	Observed	0·130	0·135	0·155	0·090	0·155
		Calc.	0·140	0·140	0·160	0·100	0·155
Total polyphenols	380	Observed	0·55	0·55	0·56	0·53	0·56
		Calc.	0·55	0·54	0·55	0·54	0·54
Total colour	460	Observed	0·43	0·42	0·44	0·40	0·43
		Calc.	0·43	0·40	0·44	0·39	0·41

Standards

A bulk of each standard tea was acquired and used over a period of six months. During this time replicate determinations of polyphenols were carried out periodically by the manual method. These showed no change in the average values over this time.

Application to tea

Replication of peaks has been found to be satisfactory and the resolution has been found adequate at a sampling rate of 40 samples/h. Total colour and total polyphenols have, on occasion, been run at 60 samples/h with satisfactory resolution.

The proportion of each constituent tea in a number of blends was known and this enabled the peak heights of the blend infusions to be calculated from the data on the individual teas. Table IV gives the observed and the calculated peak heights. Although the polyphenol content of the individual teas varied widely Table IV shows that the values for the blends are predictable from the values for the component teas.

Conclusions

The methods described are comparative only and the interpretation of the results depends upon suitable calibration. So far, teas whose characteristics have been evaluated by a manual method have been used for calibration purposes. This is not an ideal procedure, and it is hoped that in the future it may be possible to use pure theaflavin as a standard for this part of the work. In the meantime the methods devised provide a rapid and convenient tool for quality control purposes. Their usefulness would be increased if an automatic method of preparing the tea infusions was worked out. One possible way of doing this would be to disperse leaf tea in cold water using, for example, the Technicon Solid-Prep Sampler, to heat the dispersion within the manifold, to remove the suspended leaf by means of a dialysis unit and then to proceed as described in this paper. No work has so far been done on these lines; its success would depend upon comminuting the leaf tea (without altering its characteristics) to the point where a uniform suspension could be prepared.

Crispin, Payne & Swaine[4] have commented in a recent publication that the Roberts & Smith method is not suitable for development as an automatic procedure. This is true of the method as it stands but the modifications introduced have enabled workable methods to be developed.

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SENSORY AND OBJECTIVE MEASUREMENTS OF THE QUALITY OF FROZEN STORED HADDOCK OF DIFFERENT INITIAL FRESHNESSES

By J. J. CONNELL and P. F. HOWGATE

The quality of haddock fillets cut from fish held for different periods in melting ice and then stored in the frozen state at three different temperatures has been assessed on the same samples by a taste panel and by a series of objective tests. The measured changes in the texture and flavour of the fish were compared with changes in objective parameters in order to determine the value of the latter in predicting eating quality. The results for fish caught in the North Sea show that haddock fillets keep less well than cod fillets during frozen storage.

Introduction

In a previous publication[1] the results were reported of a series of sensory and objective tests on frozen cod of different qualities. These results allowed certain conclusions about the usefulness of the objective tests of quality to be made. For the sensory tests a specially developed taste panel scoring system was employed. As objective measures of quality before freezing, determinations of the concentrations of trimethylamine and hypoxanthine were used. Objective quality loss brought about by freezing and frozen storage was measured by determinations of extractable protein, cell fragility and colour ratio. In addition, measurements of pH were carried out because textural eating quality depends on this parameter to a certain extent.

The present paper describes results of a similar kind obtained on haddock.

Experimental

Material

The storage tests described in this paper were carried out on North Sea haddock (*Gadus aeglefinus* L.), 35–40 cm long, caught by trawling approximately 50 miles off Aberdeen throughout the period May, 1966–June, 1967. The fish were gutted immediately after being caught and were packed in melting ice. After periods of 2, 5, 8, 14 and 17 days in ice the fish were filleted, and the fillets were frozen in an air blast or plate freezer at −34°. The frozen fillets were closely wrapped in aluminium foil to prevent desiccation and stored at temperatures of −14°, −22° or −29°. Each bundle contained fish of only one icing treatment. The samples were thawed overnight in still air at 1·5°.

For direct estimation of the effect of freezing and thawing alone the 'paired fillet' technique was used – one fillet from a fish was frozen and thawed overnight without intervening storage and compared with its pair held at 0°.

The sampling distribution is shown in Table I.

TABLE I

Numbers of samples analysed after various treatments

Freezing treatment	Days in ice				
	2	5	8	14	17
Stored at −14°C	34	30	30	16	18
,, −22°C	16	16	15	11	9
,, −29°C	7	10	7	7	4
Frozen and thawed without storage	4	2	2		2
Not frozen	8	4	6	2	4

Sensory methods

The method of cooking and presentation to the taste panel was as described for cod[1] with the exception that the cooking time was reduced to 25 minutes. This was done because the haddock samples were thinner than the cod samples.

12 samples were presented in a session, a total of 264 samples being tasted in 22 sessions spread over 6 weeks. Samples were presented to the panel in random order.

In the case of frozen cod[1] it was necessary to develop a new scoring system,[2] and with frozen haddock, a suitable scoring system had similarly to be developed. The system for haddock resembles in principle that described for cod in that spoilage in the period before freezing was assessed separately from the changes occurring during frozen storage. Assessment for spoilage before freezing was carried out using 10-point scales for freshness odour and freshness flavour. The odour and flavour descriptions in this 10-point scale are similar but not identical to those given in the 11-point scale for spoiling of unfrozen cod.[3] In the haddock scale, scores of 10 and 1 correspond to absolutely fresh and putrid, respectively. It was found that the odour, flavour and textural deteriorative changes occurring in frozen stored haddock are very similar to those occurring in frozen stored cod; in particular the accumulation of the characteristically unpleasant cold-storage odour and flavour occurred regardless of the initial freshness of the haddock. For cold-storage odour and flavour, the extreme scores of 0 and 5 denote 'absent' and 'very strong', respectively; in the scale for firmness, 0 and 6 denote 'very soft' and 'extremely tough', respectively, a score 2 representing the average firmness of unfrozen cod of average pH caught in the North Sea; in the scale for dryness, 0 and 4 denote 'sloppy, watery' and 'extremely dry', respectively, a score of 1 representing the average dryness of unfrozen cod of average pH caught in the North Sea. Anchoring of the normal firmness and dryness scores in terms of the values for cod rather than for haddock was done for two reasons. The first was that the panel employed for haddock were already familiar with the textural attributes of cod and could readily transpose; the second is that constant reference scale points related to a single species are provided, thus avoiding a multiplicity of reference points for the normal textural attributes of different species. This means that the textural scores for normal average unfrozen haddock (or other species) are not necessarily the same as those for normal average unfrozen cod.

In addition to the scores for separate attributes, an assessment of the overall acceptability was obtained using the 9-point hedonic scale in which scores of 9 and 1 denote 'like extremely' and 'dislike extremely', respectively.

In all cases the reported scores are the mean panel scores.

Objective methods

Determination of the concentrations of trimethylamine[4] and hypoxanthine[5] were chosen as tests known to be related to sensory changes occurring during iced storage of haddock. In the same way as was done for cod,[1] determinations of cell fragility and colour ratio were used as measures of changes occurring during frozen storage.

Because of its effect on firmness, dryness and cell fragility, the pH of each sample was measured on a homogenate prepared by blending 10 g of fish in 100 ml of water. This ratio is less than in the case of cod (50 g in 100 ml) because the amount of sample available was less. The measured pH of such homogenates depends to a certain extent on the ratio of fish to water and in order that the pH of the cod and haddock samples may be compared properly, this effect has to be taken into account. Experiments with different ratios of the same sample of fish gave the following relationship:

$$\Delta \text{ pH} = 0{\cdot}089 \left(\frac{\text{ml water}}{\text{g muscle}}\right)^{\frac{1}{4}}$$

where Δ pH = pH of diluted sample − pH of undiluted sample. This relationship holds for fish of a wide range of pH values. Thus, for comparison under the same conditions of dilution, 0·16 units need to be subtracted from the pH values of the haddock.

Experimental details of these objective methods have been given.[6]

Extractable protein determinations were carried out on 124 samples; the other objective determinations were carried out on all 264 samples.

Statistical methods

A total of 16 variables were recorded for each sample with designations as follows:

Month caught (January 1966 = 0)	*M*	Dryness	*Dr*
		Overall acceptability	*OA*
Days in ice	*D*	pH	pH
Weeks of frozen storage	*W*	Colour ratio	*CR*
Freshness odour	*FO*	Cell fragility	*CF*
Freshness flavour	*FF*	Protein extractability	*PE*
Cold-storage odour	*CSO*	Trimethylamine (mg N/100 g fish)	TMA
Cold-storage flavour	*CSF*		
Firmness	*F*	Hypoxanthine (μM/g fish)	Hy

The interrelationships between variable were examined by means of their correlation coefficients and multiple regression analysis. Where exponential curves were fitted to data, the maximum likelihood method was used as described by Stevens.[7] Most of the computation was carried out on an Elliot 803 B digital computer using a standard library programme for the multiple regressions and a specially written one for the exponential regressions. Throughout this paper the symbols **, *, and n.s. represent values which are significant at $P < 0{\cdot}01$, $P < 0{\cdot}05$ and not significant, respectively. The abbreviations r and d.f. mean correlation coefficient and degrees of freedom, respectively.

Results and Discussion

Effects of freezing and thawing alone

Sensory measurements

The results of measurements on paired fillets from 10 fish iced for periods of 2, 5, 8 and 17 days are shown pooled in Table II.

There is an indication that the changes in freshness flavour, cold-storage flavour, and acceptability are less for the 2 fish stored for 17 days in ice than for the others, but there are insufficient data in these comparisons for more detailed examination of the effect of icing to be made.

These results show that, as with cod, a taste panel can readily detect changes in sensory attributes produced by freezing and thawing in this manner. In the cod experiments rapid thawing caused a smaller change than thawing overnight at 1·5° but this point was not investigated with the haddock.

Objective methods

8 comparisons of paired fillets iced for 2, 5, 8 and 17 days were made with respect to trimethylamine and hypoxanthine concentrations and 7 comparisons with respect to cell fragility (*CF*) and protein extractability (*PE*).

A significant ($P = 0{\cdot}01$) fall of 5·9% in *PE* was observed; all other changes were not significant. Similar observations were obtained on cod treated in this way.[1]

Changes occurring during frozen storage—changes in sensory measurements

Freshness scores

Fillets stored at −14° were sampled at 1 or 2 week intervals up to 3 months, and thereafter at monthly intervals up to 12 months from the commencement of storage. For fish iced for 2 and 5 days, *FF* falls slightly over the first 6 weeks and then remains constant; the corresponding values for fish iced for 8 and 14 days remain constant throughout and the values for 17 days increase slightly during the first 10 weeks and remain constant thereafter. Similar behaviour was noted for cod.[4] The magnitude of the initial changes in haddock stored at −14° are shown in Table III. Storage at this temperature significantly reduced the overall range of *FF* scores; after 8 weeks' storage haddock iced for 2 and 5 days cannot be distinguished on the basis of *FF*. The *FF* scores of haddock iced for 2 and 5 days and then stored for longer than 8 weeks at −14° are lower by 1·3 and 0·9 units, respectively,

TABLE II

Effect of freezing and thawing without intermediate storage on sensory attributes of haddock of different initial freshnesses

Variable	Mean-score differences†
Freshness flavour	−0·490*
Cold-store flavour	0·249*
Firmness	0·191**
Dryness	0·269*
Acceptability	−0·750*

† Difference calculated as follows: score of fillets frozen and thawed − score corresponding fillets which are left unfrozen.

TABLE III

Freshness flavour scores of haddock stored at −14°C

Days in ice before freezing	Weeks in store	Number of samples	Mean score	Mean score of haddock frozen and thawed without storage
2	⩾8	19	6·78	7·54
5	⩾8	21	6·81	7·24
8	⩾1	30	6·61	6·68
14	⩾1	14	4·82	4·78
17	⩾8	11	4·48	3·44

than the *FF* scores of corresponding unfrozen samples; these differences are almost exactly the same as those found for cod.[1]

Fillets stored at −22° and −29° were sampled at approximately 3, 6, 9 and 12 months, and at 6 and 12 months, respectively. For each icing treatment linear regressions of *FF* on weeks of storage were calculated separately. In no case was the regression coefficient significant, showing that at the two lower temperatures of frozen storage *FF* does not change up to 12 months for the haddock of the icing treatments examined. This enabled more information about the effects of freezing and thawing alone to be obtained. Comparison of the pooled results at −22 and −29° with the corresponding *FF* of unfrozen haddock showed that freezing and thawing had little if any effect on *FF* of fish iced for 14 and 17 days but reduced the *FF* of that iced for shorter periods by about 0·43 units, in agreement with the value obtained from the paired-fillet experiment described above.

Freshness odour scores (*FO*) were highly correlated with *FF*:

$$FO = 0{\cdot}91\ FF + 0{\cdot}51 \text{ (r} = 0{\cdot}980 \text{ with 262 d.f.)}$$

Cold-storage deterioration scores

The score for cold-storage flavour (*CSF*) of unfrozen fish should, of course, be zero. However, because the unfrozen fish was distributed randomly among many samples having pronounced *CSF*, occasional samples of the former were given false positive scores for this attribute. This resulted in a mean *CSF* of 0·13 being recorded for unfrozen fish. The mean *CSF* of all samples of haddock frozen and thawed without storage (including those from the paired fillet experiment) was 0·36. The values 0·13 and 0·36 are significantly different, confirming that freezing and thawing alone can result in a significant increase in *CSF*.

The change of *CSF* with storage time at −14° is shown in Fig. 1. The values increase in a curvilinear manner and become asymptotic to the time axis. It was found that an exponential first-order curve fitted the data for fish iced for 2, 5 and 8 days, the residual deviations from the calculated lines having a symmetrical distribution. The calculated coefficients in the exponential regressions for the fresher fish are shown in Table IV. There is no regular effect of icing on the coefficients, and none of the coefficients when taken in pairs is significantly different. It is therefore concluded that freshness up to 8 days in ice is without effect on the development of *CSF* in haddock under these conditions. With cod subjected to the same icing treatment there are indications that the rate of development of *CSF* is higher in the freshest fish. The curve shown in Fig. 1 for the fresh fish is obtained using the pooled data from fish iced for 2, 5 and 8 days. For the data on fish iced for 14 and 17 days linear regressions are satisfactory as shown in Fig. 1; in such stale fish either the rate of development of *CSF* is low or the taste panel have difficulty in detecting *CSF* when it occurs together with iced spoilage off-flavours.

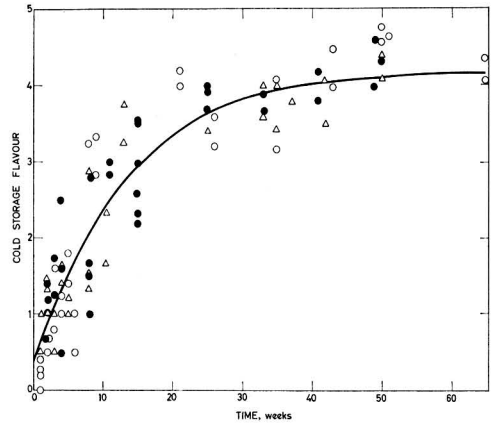

FIG. 1. *Changes in cold-store flavour scores of iced haddock during storage at −14°C*
Fish stored initially for the following days in ice:
○ 2, ● 5, △ 8

TABLE IV

Change of cold-storage flavour during storage of haddock at −14°C
Exponential regression: $CSF = a + be^{-kw}$

Days in ice	*a*	*b*	*k*
2	4·31	−4·52	0·106
5	4·73	−3·90	0·0442
8	3·97	−3·51	0·0727
2, 5 and 8	4·23	−3·90	0·0748

At −22°, *CSF* of haddock iced for 2, 5 and 8 days increases significantly with time but the regressions for the different periods are not significantly different. The following is the regression for the pooled data on fish iced for 2, 5 and 8 days at this temperature:

$$CSF = 0{\cdot}91 + 0{\cdot}0436^{**}\ W$$

The *CSF* of fish iced for 14 and 17 days do not change significantly at −22°.

There is no significant difference between the mean *CSF* of haddock held for 6 and 12 months at −29°. However, when the scores for fish iced for 14 and 17 days are left out, the mean *CSF* of samples stored at this temperature is 1·024 which is significantly higher than the corresponding mean score of fish frozen and thawed without storage. It is concluded, therefore, that *CSF* of haddock does increase slightly over 1 year's storage at −29° in contrast with the behaviour of cod.[1] With cod, *CSF* is related to the pH of the fish, but no such effect was observed with haddock.

Cold-storage odour scores (*CSO*) were highly correlated with *CSF*.

$$CSO = 0{\cdot}755\ CSF - 0{\cdot}081 \text{ (r} = 0{\cdot}88 \text{ with 262 d.f.)}$$

It has been demonstrated[1] that the firmness (*F*) of cod is affected by pH and this is also true for haddock. For unfrozen haddock samples the relationship is:

$$F = 14{\cdot}82 - 1{\cdot}83\ \text{pH}$$

F of stale fish whether frozen or unfrozen is generally lower than that of fresher fish. This observation is entirely accounted for by the fact that the pH of iced fish increases during icing.

In the following discussion, all F scores have been corrected to a nominal pH of 6·70, (the mean value for samples of all icing treatments), using the regression coefficient quoted above for unfrozen samples. Thus, any effects described are not attributable to variations in sample pH.

At −14°, F increases exponentially with time and, unlike the situation with cod, there is no difference between the separate icings (Fig. 2). The pooled regression equation is:

$$F = 4{\cdot}61^{**} - 1{\cdot}84^{**} \exp(-0{\cdot}076^{**}W)$$

The extrapolated value of F at zero time is 2·77, which is not significantly different from the value of 2·69 for the fillets that were frozen and immediately thawed.

As the increase in F of haddock at −14° was similar to that of cod, it was expected that, at −22°, F for haddock would increase with storage time in the same way as for cod. However, surprisingly, it was found that for haddock the regression of F on storage time was not significant, even though the mean F of all samples stored at this temperature was the high value of 3·68. The reason for this behaviour is shown in Table V in which the data are grouped over three ranges of storage time; the adjacent pairs of F scores are significantly different from one another. It appears that F for the longest storage times is much lower than anticipated. This effect is attributed to F changing with season in a way which is independent of seasonal changes in pH. It would appear that the fish shown in the second (and possibly third) group of Table V, i.e. fish caught between September and December, were either intrinsically firmer than those of the first group, caught between February and May, or exhibited a faster rate of increase of F with storage. If the assumption is made that the seasonal variation is cyclical with a period of one year, then the samples in the third group can be compared with those at zero time of storage. When this is done it is found that for haddock an increase of 0·83 F units occurs during storage at −22° for 1 year.

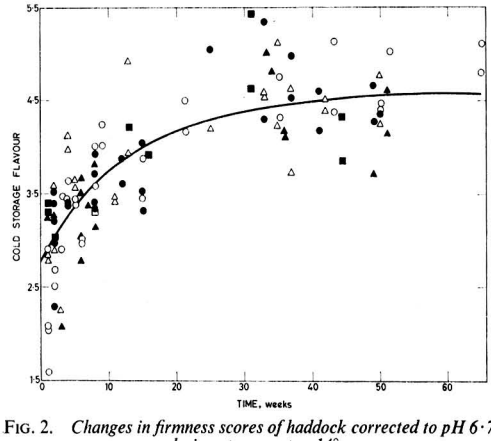

FIG. 2. *Changes in firmness scores of haddock corrected to pH 6·70 during storage at −14°C*

Fish stored initially for the following days in ice:
○ 2, ● 5, △ 8, ▲ 14, ■ 17

TABLE V

Firmness scores of haddock during storage at −22°C

Mean storage time, weeks	Range, weeks	Number of samples	Mean firmness score
12·8	9–16	15	3·35
31·0	24–38	26	3·97
50·9	43–56	22	3·52

A similar seasonal effect on F is also apparent in fillets stored at −29°. Thus, the mean values of F of samples stored for 6 months and 12 months are 3·44 and 3·05, respectively; the difference is significant at the 5% level. In addition, the difference between the F value of samples stored for 12 months and that of samples frozen and immediately stored, namely 0·36, is significant at the 5% level. There is therefore strong evidence that F increases during storage at −29°. In addition, fish examined after 6 months (caught in December) are firmer for reasons similar to those given above for samples stored at −22°.

A similar seasonal effect should influence the F scores of fillets stored at −14° but the shape of the curve (Fig. 2) makes isolation of the effect difficult.

Dryness (Dr) is very closely related to F:

$$Dr = 1{\cdot}58 + 0{\cdot}283^{**}F \quad (r = 0{\cdot}92 \text{ with } 262 \text{ d.f.})$$

Because of the high correlation between F and Dr the effects of processing and season on Dr are qualitatively the same as on F.

Overall acceptability scores (OA)

It should be emphasised that these scores are independent and personal expressions of the opinions of individual panel members about the degree of desirability of the samples. The results here are therefore strictly pertinent only to the group of persons making up the panel and to fish cooked and eaten in the way described. However, unpublished comparison between the OA of the panel used and a wide cross-section of ordinary consumers show that the responses of the two groups are qualitatively similar. OA scores of fish iced for 2, 5 and 8 days stored at −14° decrease exponentially with time, the computed regressions of the form, $y = a + b \exp(-kW)$ being:

$$2 \text{ days: } OA = 3{\cdot}15 + 3{\cdot}36 \exp(-0{\cdot}16\ W)$$
$$5 \text{ days: } OA = 2{\cdot}92 + 2{\cdot}66 \exp(-0{\cdot}06\ W)$$
$$8 \text{ days: } OA = 3{\cdot}39 + 2{\cdot}10 \exp(-0{\cdot}11\ W)$$

The asymptotic values, a, and exponential coefficients, k, are not significantly different from one another. The amounts of change, b, show a systematic dependence on period stored in ice, and the values obtained for fish iced for 2 and 8 days are significantly different. This is because the starting values, $a + b$, when $W = 0$ are dependent upon icing treatment. There is no change during storage of the scores for fish iced for 14 days and 17 days, and the mean scores are 2·85 and 2·07, respectively.

During storage at −22° OA decreases linearly to the limit of the experiment and the regression equations are as follows:

$$2 \text{ days: } OA = 6{\cdot}32 - 0{\cdot}0450^{**}W$$
$$5 \text{ days: } OA = 5{\cdot}56 - 0{\cdot}0245^{**}W$$
$$8 \text{ days: } OA = 5{\cdot}39 - 0{\cdot}0256^{**}W$$

The regression coefficient for the fish iced for 2 days is significantly different from that for either 5 days or 8 days. With cod stored at −14° and −22°, the rates of fall of OA decrease systematically with icing, but this behaviour is not so apparent with haddock stored under the same conditions. At −22° there is no effect of storage on the haddock stored for 14 or 17 days, and their mean scores are 2·60 and 2·09 respectively.

At $-29°$ there is no effect of storage for any icing treatment. The mean values for 2, 5, 8, 14 and 17 days in ice are 6·33, 5·84, 5·24, 2·96 and 1·95, respectively.

Changes in objective measurements

Trimethylamine and hypoxanthine concentrations

It was found that for any icing treatment no significant change in the concentration of TMA occurred during frozen storage at any temperature. The same observations were made on cod samples of the same type treated in the same way.[1] Castell *et al.*[8] have recently observed with uniform batches of very fresh cod that small but significant increases in TMA occur during frozen storage conditions similar to those described here. The failure to observe similar increases in the present experiments on cod and haddock is possibly due to the use of much less homogenous and less fresh starting material for freezing. In consequence, both the initial TMA values of this material and probably also the subsequent rates of increase of TMA during frozen storage were more variable than in the material used by Castell *et al.*[8] Thus the small increases which probably occur in individual samples are masked in the general scatter of values.

In contrast, it was found that for all icing treatments the Hy content of the fillets increased during storage at $-14°$; no systematic effect of icing on the rate of increase of Hy was observed. The pooled rate of increase of Hy over all icings was at this temperature of storage, 0·0138 μM Hy/g/W. There is a small rise in Hy during storage at $-22°$ but it is not significant at the 10% level. There is no indication of a rise during storage at $-29°$. Spirelli *et al.*[9] measured the Hy content of ocean perch (*Sebastodes alutus*) during storage at $-29°$ up to 4 months and found it did not change.

pH

As with cod,[1] pH increases in a quadratic manner with days in ice. In addition, visual inspection of the results indicated that pH was influenced by both frozen storage and season.

Insufficient data on unfrozen samples were available to determine the effect of season directly so it was necessary to use the data from frozen samples in order to separate the effects of both season and frozen storage. To measure the effect on pH of frozen storage alone, the samples (collected at the same time of year) were compared after the same period of storage, namely 12 months. In this comparison fish from all icing treatments were used, which necessitated taking into account the variations of pH due to differences in icing treatment. It was found that for the different frozen storage temperatures, the variations of pH with D^2 were not significantly different, and therefore a pooled regression coefficient connecting pH with D^2 was calculated (0·00246). Using this pooled coefficient, the following regressions were obtained:

$$\text{not frozen: pH} = 6{\cdot}517 + 0{\cdot}00246\ D^2$$
$$\text{stored at } -29°\text{: pH} = 6{\cdot}496 + 0{\cdot}00246\ D^2$$
$$\text{stored at } -22°\text{: pH} = 6{\cdot}453 + 0{\cdot}00246\ D^2$$
$$\text{stored at } -14°\text{: pH} = 6{\cdot}337 + 0{\cdot}00246\ D^2$$

There is a consistent fall in regression constant as one passes from 'not frozen' to 'stored at $-14°$'. The constants for 'not frozen', 'stored at $-29°$' and 'stored at $-22°$' are not significantly different, but that for 'stored at $-14°$' is significantly different from the rest, indicating a real fall of about 0·18 pH units during 12 months' storage at $-14°$

The effect of season was determined by using the data for fish stored at $-22°$ and $-29°$, assuming that storage by itself at these temperatures had a negligible effect on pH. The pH values at discrete periods of frozen storage, and therefore of month caught, were corrected to a notional 2 days in ice using the regression coefficient quoted. The mean corrected pH values plotted against month caught are shown in Fig. 3. There is a regular change of pH with season which like that of cod[1] is presumably cyclic in nature and dependent upon the nutritional status of the fish at different times of the year.

Cell fragility (CF)

The change in CF with time of storage at $-14°$ is shown in Fig. 4. The values for samples iced for 2, 5 and 8 days decrease in an exponential manner but there is no significant change in the results for the samples iced for 14 and 17 days. Separate regressions were calculated for each of the former group, but they were not significantly different. The pooled regression, shown in Fig. 4 is as follows:

$$CF = 0{\cdot}301 + 0{\cdot}687 \exp(-0{\cdot}253\ W)$$

During storage at $-22°$ CF decreases, and over 1 year a linear regression adequately represents the data; the values are also dependent upon pH as follows:

$$CF = 3{\cdot}63 - 0{\cdot}389^{*}\ \text{pH} - 0{\cdot}0116^{**}W$$

There is no significant change during storage up to 1 year at $-29°$ but the values are dependent upon pH as follows:

$$CF = 6{\cdot}10 - 0{\cdot}776^{**}\ \text{pH}$$

At all three temperatures of storage, the sample-to-sample variation resembled that given for cod.[1]

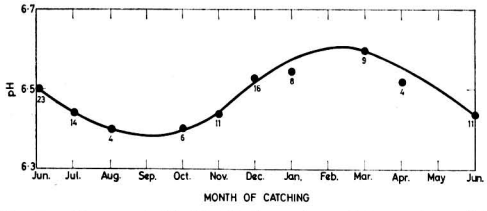

FIG. 3. *Change of pH of haddock caught at different times of the year*

The pH measurements have been corrected to a notional 2 days in ice. Each point is the mean of the number of samples given below it.

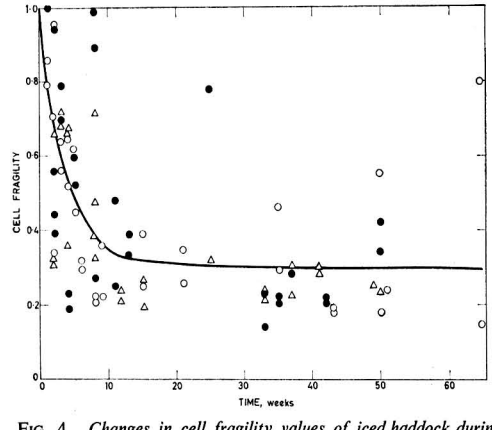

FIG. 4. *Changes in cell fragility values of iced haddock during storage at $-14°$C*

Fish stored initially for the following days in ice:
○ 2, ● 5, △ 8

Colour ratio (CR)

A slight increase in *CR* was observed during storage at −14°, and very little change at the lower temperatures of storage. The results are not recorded here because they show that *CR* could not be used as an objective method for measuring storage deterioration of frozen haddock.

Protein extractability (PE)

The change in *PE* with storage time at −14° is shown in Fig. 5, the calculated regression being as follows:

$$PE = 18{\cdot}0 + 54{\cdot}9 \exp(-0{\cdot}0264\ W)$$

At −22° the calculated linear regression is as follows:

$$PE = 85{\cdot}9 - 0{\cdot}755\ W$$

At −29° only samples stored for 12 months were available and the mean value at this temperature was 71·3%. This value is just significantly different from the mean of the samples that were frozen and immediately thawed (80·3%), and indicates that a small drop in *PE* occurs during 1 year's storage at −29°.

As with *CF*, the sample-to-sample in *PE* for haddock at all temperatures of storage resembled that given for cod.[1]

Correlations between variables

As with the cod experiment, certain correlations between the variables in the haddock experiment were examined with the main aim of finding how well the objective tests can predict the subjective assessments. In the final comparisons in this section, relationships between overall acceptability and the individual quality attributes of freshness flavour, cold storage flavour and firmness are examined; here the aim is to discover how acceptability depends upon the other subjective assessments of quality taken individually. It must be remembered that the correlations and particularly the prediction equations are true only for the fish examined and may not be the same for haddock of other sizes or from other fishing grounds.

Freshness flavour (*FF*) and trimethylamine or hypoxanthine

TMA does not change during frozen storage or only very slightly, and so it can be used to predict the original *FF* of the fish before freezing almost as accurately as it can for unfrozen fish. On the other hand, Hy does change significantly on frozen storage and therefore cannot unambiguously predict the original *FF* before freezing for all conditions of frozen storage.

In addition, *FF* changes under some conditions of frozen storage so any attempt to predict this subjective evaluation from any objective method using fish of unknown frozen storage will be subject to a further error. Despite these restrictions, the highest correlations found between *FF* and various ways of treating the TMA and Hy data were similar to those found for cod.[1] For the haddock data, the highest correlation is −0·852 (240 d.f.) for *FF versus* TMA, followed by −0·823 (240 d.f.) for *FF versus* Hy. For all conditions of icing and frozen storage, the following relationship was obtained:

$$FF = 7{\cdot}20 - 0{\cdot}125\ \text{TMA}$$

Cold storage flavour (*CSF*), firmness (*F*) and cell fragility (*CF*)

The relationships between *CSF* and *CF*, and corrected *F* and *CF* for fish iced for 2, 5 and 8 days are shown in Figs 6 and 7 respectively. The reasons why the other samples are not included in this comparison are the same as those

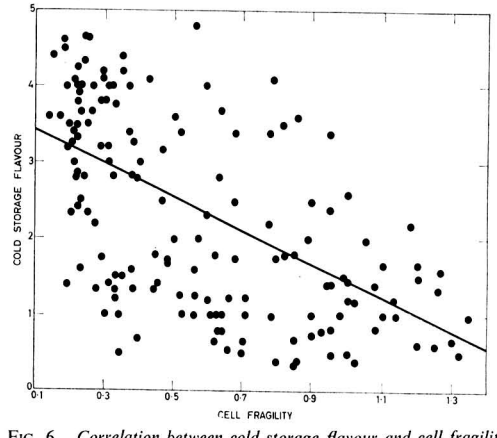

FIG. 6. *Correlation between cold storage flavour and cell fragility values for haddock stored initially for 2, 5 or 8 days in ice and held under all frozen storage conditions*

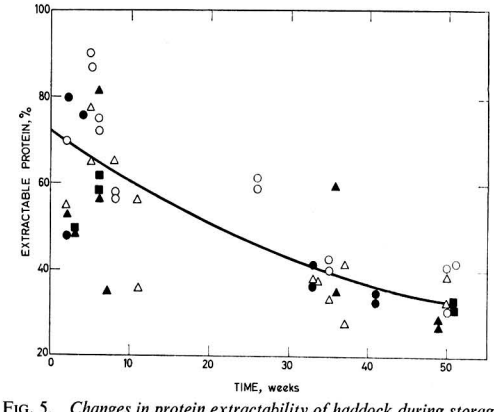

FIG. 5. *Changes in protein extractability of haddock during storage at −14°C*

Fish stored initially for the following days in ice:
○ 2, ● 5, △ 8, ▲ 14, ■ 17

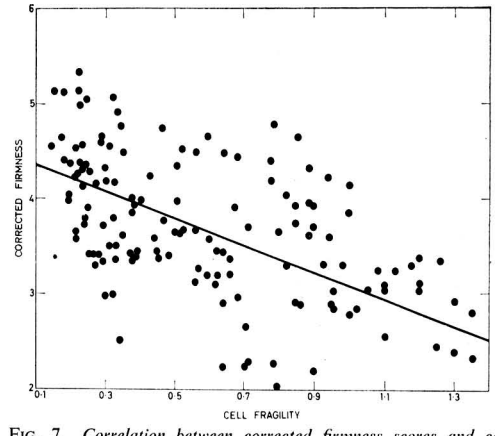

FIG. 7. *Correlation between corrected firmness scores and cell fragility for samples treated as for Fig. 6*

expressed when considering the identical comparisons for cod.[1] The correlation coefficients between *CSF* and *CF* and between *F* and *CF* are 0·55 (154 d.f.) and 0·45 (154 d.f.) respectively. In both instances inclusion of a term for pH makes a highly significant reduction in the residual variance, the multiple regression coefficient in both cases being 0·71 (153 d.f.). The lines shown in Figs 6 and 7, respectively, are obtained from the following linear relationships:

$$CSF = 27{\cdot}4 - 2{\cdot}15^{**}\ CF - 3{\cdot}63^{**}\ \text{pH}$$
$$F = 23{\cdot}4 - 1{\cdot}18^{**}\ CF - 2{\cdot}86^{**}\ \text{pH}$$

for pH values of 6·56, (the mean value of the samples in this set) and 6·70 respectively.

Linear regressions are probably not the best models to use but other simple ones such as reciprocal or logarithmic functions did not reduce the residual variance by a significant amount from the linear model. This point was also noted for the cod samples.[1]

Cold storage flavour (*CSF*), firmness (*F*) and protein extractability (*PE*)

The relationships between *CSF* and *PE* and corrected *F* and *PE* for samples iced for 2, 5 and 8 days are shown in Figs 8 and 9, respectively. The correlation coefficients between *CSF* and *PE* and between *F* and *PE* are 0·77 (73 d.f.) and 0·73 (73 d.f.) respectively. In both instances inclusion of a term for pH makes a significant reduction in the residual variance, the multiple regression coefficients being 0·81 (72 d.f.) and 0·83 (72 d.f.) for the relationships involving *CSF* and *F*, respectively. The lines shown in Figs 8 and 9, respectively, are obtained from the following best-fitting relationships:

$$CSF = 18{\cdot}2 - 0{\cdot}0518^{**}\ PE - 1{\cdot}97^{**}\ \text{pH}$$
$$F = 16{\cdot}6 - 0{\cdot}0289^{**}\ PE - 1{\cdot}68^{**}\ \text{pH}$$

using the same pH values as described above.

Overall acceptability (*OA*) freshness flavour (*FF*) cold-storage flavour (*CSF*) and firmness (*F*)

For unfrozen haddock *OA* is very closely determined by *FF* as follows:

$$OA = 1{\cdot}17^{**}\ FF - 2{\cdot}31\ (r = 0{\cdot}993,\ 22\ \text{d.f.})$$

Thus, for the group of tasters examined, natural variations in *F* of unfrozen fish (which is the other main quality attribute recorded) is of negligible or very minor significance in influencing its acceptability. However, for frozen stored fish, where the variations are, of course, much larger, *F* as well as *CSF*, has a large influence on *OA*. Regardless of the *F* and *CSF* values for frozen fish, *FF* strongly influences *OA* as indicated by the highly significant correlation coefficient of 0·825 between *OA* and *FF*. Inclusion of terms for *F* or *CSF* in a multiple regression increases the correlation coefficient as follows:

$$OA = 0{\cdot}145 + 1{\cdot}09^{**}\ FF - 0{\cdot}787^{**}\ F$$
$$(r = 0{\cdot}931,\ 208\ \text{d.f.})$$
$$OA = 1{\cdot}05^{**}\ FF - 0{\cdot}586^{**}\ CSF - 1{\cdot}44$$
$$(r = 0{\cdot}941,\ 208\ \text{d.f.})$$

Thus, as far as the panel's judgment of acceptability is concerned, the deleterious effects of poor processing are additive.

Selection and use of objective methods for quality assessment of frozen haddock

The considerations which apply in the selection of objective tests for measuring the quality of frozen haddock are essentially the same as those discussed previously for cod.[1] In the case of frozen haddock, determinations of either TMA or Hy offer nearly equally good predictions of flavour changes resulting from initial differences in icing treatment, with perhaps TMA being very slightly superior. Protein extractability determinations are able to predict deteriorations resulting from frozen storage much better than *CF* determinations; as noted above, *CR* cannot be used for haddock.

In principle, the acceptability, to a consumer or group of consumers, of frozen fish of any quality can be predicted within the limits of error from objective measurements if the relevant relationships are known. For example, under the present conditions, *OA* can be found from the following relationship which accounts for 62·6% of the variance:

$$OA = 2{\cdot}46 - 0{\cdot}0789^{**}\ \text{TMA} + 0{\cdot}0372^{**}\ PE$$

Judged from the results of all subjective measurements, the quality of frozen haddock deteriorates faster than that of cod stored under the same conditions. On the other hand, the objective measurements of *PE* and *CF* change during frozen storage of haddock more slowly than they do in the case of cod stored under the same conditions.

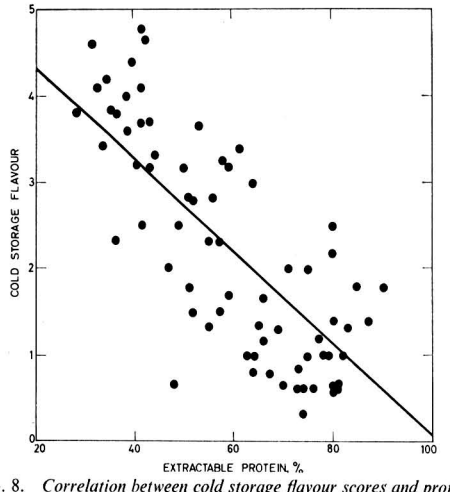

FIG. 8. *Correlation between cold storage flavour scores and protein extractability for samples treated as for Fig. 6*

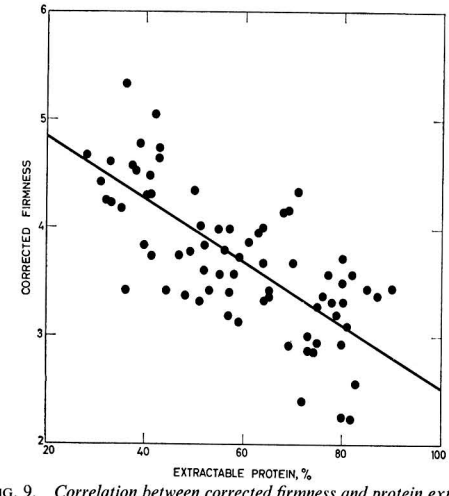

FIG. 9. *Correlation between corrected firmness and protein extractability for samples treated as for Fig. 6*

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EVALUATION OF WHISKY DISTILLERY BY-PRODUCTS

I.—Chemical composition and losses during transport and storage of malt distiller's grains

By T. B. MILLER

Samples of wet malt distiller's grains (*MDG*) have been analysed to assess the quality of the material and the variation between the effects of distilleries on quality and the losses incurred during transport and farm storage.

Comparative analyses of *MDG* and the parent barley showed that the recoveries of soluble carbohydrate, protein, holocellulose, lipid and minerals in the *MDG* and malt culms amounted to 16, 54, 90, 95 and 32% respectively, which indicates a depletion of soluble carbohydrate and minerals (particularly potassium) and a concentration of structural components and lipid.

A survey of the material leaving 12 different distilleries over a period of 6 weeks showed little difference in composition between distilleries. Transport losses were negligible.

Losses of dry matter during storage ranged from 11 to 21% depending on length of storage. The addition of salt did not result in a consistent reduction in these losses.

Introduction

Whisky distilleries in Scotland are either 'malt' or 'grain' types. In the former, barley malt forms the sourceof enzymes and the substrate for subsequent fermentation. The substrate in grain distilleries is fortified by the addition of unmalted cereals such as barley, maize, wheat, oats or rye.

Malt distilleries are distributed throughout the north and north-east counties of Scotland and, with a few exceptions, all the grain types are located in the south of Scotland and in England.

The by-products of distilleries vary according to the type of distillery. Of these by-products the residue after extraction of the malt which is referred to as 'distillers grains', is quantitatively the most important. In the malt distilleries, malt distiller's grains (*MDG*) are represented by the insoluble constituents of the malted barley only, whilst that of grain distilleries contains about 15% barley malt constituents and the remainder consists of the residues from the unmalted cereals. The quality of *MDG* also depends on the malting and extraction process which the malt undergoes in the distillery. In malt distilleries, this process is more exhaustive than that of breweries in order to ensure that the maximum amount of starch is made soluble and utilised for alcohol fermentation.[1]

The *MDG* are produced in large quantities from distilleries in the north-east counties of Scotland. The bulk of the material is sold direct from the distilleries as it leaves the mash tun. In this state the *MDG* contains about 75% of water and is referred to locally as 'draff'. The wet material is loaded on to open lorries and transported in quantities of up to 10 tons to farms. On the farm the wet material is ensiled to reduce contamination with moulds. On some farms where regular supplies are available at 1–4 week intervals each consignment of material is consumed before the next delivery. Long-term ensilage, by which quantities of about 100 tons are accumulated during the summer for feeding in the following winter is also used.

This somewhat crude handling of a highly perishable material results in considerable modification of the remaining fermentable constituents before the *MDG* is fed to stock. In the present paper preliminary studies are presented on the chemical composition of *MDG* and the effect of handling on certain constituents.

Experimental

Sampling

The samples of *MDG* were examined as received directly from the distilleries or after storage on the farm. The former samples are termed 'fresh' and the stored samples are referred to as 'unsalted' when no additives were used in the ensilage process or 'salted' when sodium chloride was mixed with *MDG* during ensilage.

All possible changes due to fermentation were arrested when samples were received in the laboratory by either storage at −20° or immediate drying at 105°. Analyses were done on dried milled samples with the exception of lactic acid and volatile fatty acids (*VFA*) determinations which were performed on the wet material after it had been homogenised with an UltraTurex homogeniser.

Analysis

To determine dry matter, samples were heated in an oven with a forced draught at 105° for 16 h. Proximate analysis was performed according to the routine procedures.[2] The methods of Jermyn[3] were used for holocellulose and α-cellulose estimations. Lignin was determined by the procedure of Waite *et al.*[4] Sodium and potassium were measured using an EEL (Evans Electroselenium Ltd) flame photometer. Calcium was determined by flame emission on a Unicam SP 900 flame spectrophotometer and magnesium was measured on the same instrument by atomic absorption. Atomic absorption methods were also used to estimate copper, zinc and manganese with the Unicam SP 90 atomic absorption spectrophotometer. Volatile fatty acids (*VFA*) were determined in a distillate obtained from an acidified sample of *MDG* by use of a Pye 104 gas–liquid chromatograph. The colorimetric method of Barker & Summerson[5] was used for the determination of lactic acid.

Results

Fate of barley constituents after malting and extraction

The chemical constituents of the original barley undergo extensive modification during the processing of malt. Some losses are incurred in the initial steeping and in respiration. The 'malt culms', which consist mainly of withered rootlets, and *MDG* represent the remaining constituents which are not removed in the extract or 'wort'.

The distribution of certain constituents among these losses and products is shown in Table I. The values for barley, culms and *MDG* are based on analyses and those for the 'extract' by difference. Although steeping losses are variable in composition, they amount to about 1% of the dry matter. They are composed of soluble constituents of the grain and any extraneous matter which may be washed off the surface. Respiratory losses amount to about 5% of dry matter though they can vary considerably and represent mainly a loss of the soluble carbohydrate. In Table I steeping and respiratory losses have been included with 'extract'.

About one-quarter of the dry matter and half the crude protein appear in the malt culms and *MDG*. Most of the ether-extractable material of the original barley is found in these two by-products, though a small fraction is metabolised during malting. The malting and extraction process results in an appreciable concentration of lipids in *MDG* which is significant in assessing the nutritive value of the material. The extraction removes 75–80% of the soluble carbohydrate but this fraction still constitutes about half the dry matter in the *MDG*. The structural constituents which are shown in Table I as either 'fibre' or 'holocellulose' and 'α-cellulose', are not wholly recovered in the malt culms and *MDG*; an appreciable fraction appears to have been made soluble. About 60% of the ash of the original barley is not accounted for in the malt culms and *MDG*. The soluble fraction of the ash and the major mineral constituents are considered to be removed in the extract, though some of the more soluble material will have been lost in the steeping process. The highly soluble potassium and sodium are almost completely extracted leaving *MDG* very low in these elements.

Chemical composition of *MDG* from different distilleries

Six consecutive weekly samples of *MDG* were collected from twelve different malt distilleries in the Moray Firth area. Individual samples were analysed for proximate constituents and the mean values for samples from each distillery source are given in Table II.

There were no significant differences in the levels of constituents between different groups of samples. This result indicates that distillery source is not an important factor in determining the nutritive value of *MDG*.

TABLE I

Proximate constituents and major minerals in 100 g barley and the corresponding products after malting and extraction

	Barley, g	Malt culms, g	*MDG*, g	Extract,* g
Dry matter	100·0	4·0	21·3	74·7
Protein (N × 6·25)	9·9	1·1	4·2	4·6
Fibre	5·3	0·6	3·7	1·0
Ether extract	1·8	0·1	1·6	0·1
Ash	3·1	0·3	0·7	2·1
Nitrogen-free extract (*NFE*)	79·9	1·9	11·0	67·0
Holocellulose	14·4	1·1	11·7	1·6
α-Cellulose	6·2	0·5	5·1	0·6
Ca	0·055	0·002	0·029	0·024
Mg	0·150	0·004	0·024	0·122
Na	0·020	0·001	0·002	0·017
K	0·480	0·070	0·008	0·402
P	0·370	0·020	0·075	0·275

* Including steeping and respiratory losses

TABLE II

Proximate composition of *MDG* samples from twelve malt distilleries
Mean values of 6 weekly samples from each distillery

Distillery	Dry matter, %	Percentage of dry matter				
		Protein	Fibre	Ether extract	Ash	*NFE*
1	24·5	19·7	17·1	7·6	3·6	52·0
2	23·6	19·6	17·2	7·8	3·2	52·2
3	22·6	19·8	16·6	7·6	3·4	52·6
4	22·0	20·9	16·3	8·1	3·8	50·9
5	24·9	18·6	18·7	8·1	3·2	51·3
6	25·4	20·4	17·5	8·2	3·2	50·7
7	23·6	19·8	16·6	8·1	3·2	52·3
8	24·1	19·8	17·6	8·0	3·3	52·7
9	22·9	17·8	17·1	8·4	3·3	53·3
10	22·4	20·3	17·4	8·5	3·2	50·5
11	23·2	20·3	16·7	8·4	3·4	51·2
12	24·5	19·9	17·7	8·9	3·5	50·0
Mean	23·9	19·8	17·3	8·2	3·3	51·4
(S.E.)	(0·28)	(0·16)	(0·14)	(0·07)	(0·03)	(0·16)

The levels of major minerals from the same set of samples are given in Table III.

Again there was no significant difference between samples from different sources except that some distilleries showed values for potassium which were significantly different ($P < 0.05$) consistently from those of other distilleries, this probably being a reflection of the potassium levels in the water of different distilleries.

Comparative analysis of fresh *MDG* and *MDG* after storage

Mean values from the analyses of fresh *MDG* and of *MDG* after storage with and without added salt are given in Table IV. The data for fresh *MDG* have been taken from Table III. The 'salted' and 'unsalted' samples were not studied in the same systematic manner and the values given for proximate constituents and minerals are means for about 30 samples received in this laboratory over two years in connexion with research and advisory work. These may be regarded as representative of the material as fed on the farm. Analyses of other constituents were performed on twelve samples.

TABLE III

Major mineral elements in *MDG* samples from twelve malt distilleries

Mean values in mg/100 g dry matter

	Ca	Mg	Na	K	P
1	143	122	5·4	45	394
2	98	89	7·8	25	286
3	144	111	11·3	63	337
4	136	159	10·0	27	400
5	105	99	6·0	44	281
6	107	101	6·8	28	307
7	116	120	6·0	58	362
8	117	114	7·8	22	331
9	143	105	8·2	21	321
10	137	100	8·7	21	367
11	130	133	7·8	37	386
12	140	139	10·2	37	345
Mean	124	114	7·7	36	343
(S.E.)	(6)	(7)	(0·4)	(4)	(14)

TABLE IV

Data from the analyses of samples of *MDG* in the fresh state and after storage without additives and with added salt (NaCl)

	Fresh	Unsalted	Salted
Dry matter, %	23·9	27·2	28·3
Protein (N × 6·25), % d.m.	19·8	21·4	20·0
Fibre	17·3	19·8	20·1
Ether extract	8·2	8·8	8·9
Ash	3·3	2·9	5·8
Nitrogen-free extract	51·4	47·1	45·2
Holocellulose	51·7	52·1	52·4
α-Cellulose	22·3	21·8	22·0
Lignin	5·1	5·6	5·5
Lactic acid	0·91	8·11	7·72
Acetic acid	0·67	1·13	0·64
Propionic acid	Nil	0·051	0·32
Butyric acid	Nil	0·51	0·32
Ca	0·12	0·18	0·12
Mg	0·11	0·14	0·11
Na	0·077	0·032	0·87
K	0·036	0·037	0·045
P	0·34	0·39	0·31
Mn, ppm d.m.	43·2	40·9	31·7
Cu	10·5	13·0	17·9
Zn	295	201	90

After storage there is a characteristic rise in the dry matter content owing to loss of water as the mass of wet material settles and ferments. The soluble carbohydrate fraction is reduced appreciably on storage. This change results in a slight increase in structural constituents and the production of lactic acid and volatile fatty acids as products of fermentation.

Comparison of salted and unsalted *MDG* did not reveal any marked differences in levels of the major constituents apart from an increased ash content due to the added salt. There is, however, an appreciable difference in the distribution of individual components of *VFA*; the addition of salt increased the proportion of propionic acid with a concomitant reduction in the butyric acid level.

The content of mineral elements has some important nutritional implications. In the fresh *MDG* the sodium and potassium levels are exceptionally low. This may be attributed to the high solubility of these elements in water, so that they are lost in the mashing process. Calcium is also low and the Ca/P ratio is only 0·3–0·5. Copper levels were usually higher in samples of salted *MDG*. The content of zinc in stored samples was variable; individual samples contained up to 3000 ppm, which was due to contamination from the galvanised bins in which they were stored.

Losses of *MDG* during transport

Nine truck loads of *MDG* were weighed after leaving the distilleries and after cumulative journeys of 5, 10 and 20 miles. All weights were recorded on the same weighbridge and two loads were also weighed after 40 miles. The weights of material lost during each journey were negligible (Table V).

TABLE V

Losses of *MDG* transported on open trucks

Journey, miles	Initial weight of *MDG*, kg	Weight loss		Dry matter loss, kg
		kg	%	
40	6966	169	2·4	2·09
40	7309	238	3·2	2·13
20	8930	206	2·3	2·36
20	4525	131	2·9	1·90
20	7258	194	2·7	2·36
20	7436	131	1·8	1·68
20	6318	858	1·4	1·13
20	6763	143	2·1	2·04
20	7030	112	1·6	1·45

Losses of *MDG* during storage on the farm

Approximately 100 tons *MDG* were stored in four covered pits during the summer of 1966. Salt (NaCl) was added at 20 lb/ton to two of the pits and the material was weighed into the pits by weighing the trucks before and after unloading. When the *MDG* was fed during the 1966–67 winter each quantity removed from the pits was weighed.

The weights of wet *MDG* and dry matter are recorded in Table VI. The loss of material was slightly less in the salted pits though the length of storage appeared to be a more important factor.

A similar experiment was conducted with two pits in 1967–68 when the pits were emptied over longer periods. The open surface of the mass of material was exposed to the atmosphere for up to about three months which is similar to practice on many commercial farms. The recovery figures are given in

TABLE VI

Weights of *MDG* ensiled and recoveries of material fed on the farm

	Pit 1 1967 (Salted)	Pit 2 1967 (Salted)	Pit 3 1967 (Unsalted)	Pit 4 1967 (Unsalted)	Pit 2 1968 (Salted)	Pit 4 1968 (Unsalted)
Wet *MDG* ensiled, tons	25·57	21·99	26·55	23·05	27·96	28·11
Wet *MDG* removed for feeding, tons	17·85	18·51	19·06	15·22	18·61	20·68
Wet *MDG* spoilage, tons	n.d.	n.d.	n.d.	n.d.	1·60	0·55
Dry matter ensiled, tons	6·39	5·90	6·64	5·60	6·68	6·71
Dry matter removed, tons	5·17	5·20	5·52	4·80	5·27	5·62
Recovery of dry matter, %	81·9	89·0	83·1	85·7	78·9	83·7
Period of storage, days	134–163	68–95	106–140	39–71	210–253	106–194

n.d. = not determined

the last two columns of Table VI. The salted material, which was stored for a longer period, suffered greater losses. The waste material, which was considered unsuitable for feeding, was also measured and amounted to 1·6 and 0·55 tons of wet *MDG* for salted and unsalted pits respectively.

Discussion

The malting and mashing processes are accompanied by extensive modifications of the original barley constituents. The major changes affect the starch through the action of α- and β-amylases on both amylose and amylopectin. The sugars and short-chain polysaccharides produced are almost quantitatively removed in the wort. Degradative changes affecting the structural polysaccharides are much less extensive. Aspinall & Ferrier[6] found that hemicellulose present in the barley husk is not modified during malting but 4% of the total pentosans is soluble in water and removed during the steeping process. The hemicellulose of the endosperm is considerably modified but any measurement of the net loss is confused by the synthesis of pentosan in the embryo. There is no evidence for changes in the barley husk during malting, though cellulose degradation and synthesis are also likely in the embryo tissues and the recent work of Conchie *et al.*[7] has established the existence of β-glycosidases in barley and malt. Protease and peptidase activity has also been demonstrated in malting,[8] thus rendering an increased fraction of the barley proteins soluble. The fat fraction is also affected, and Macleod & White[9] have shown that germination of barley under some conditions can cause lipolysis and depletion of fat reserves which are metabolised by the embryo.

Some of the degradative changes of malting and mashing are manifested to varying degrees in the composition of *MDG*. The levels of proximate constituents give only a vague indication of these changes, particularly with respect to the distribution of structural and non-structural carbohydrates. Some improvement is obtained by analysis for holocellulose and α-cellulose according to the method of Wise *et al.*[10] This separation is, however, still somewhat arbitrary and its shortcomings were apparent in the present comparison of the relative content of lignin, holocellulose and α-cellulose in *MDG* with that in barley; increases of 4·8, 3·6 and 2·8 times, respectively, were apparent. Since the difference between holocellulose and α-cellulose is mainly hemicellulose, these results imply that cellulose was dissolved to a greater extent than hemicellulose. It is more likely, however, that the α-cellulose fraction contains polymers other than β-glucosides such as pentosans which would normally appear as hemicelluloses. Moreover, the degradative changes of malting and mashing are likely to alter the partition of polysaccharides obtained in any extraction system, and without characterisation of the sugar residues, the recovery of barley constituents in *MDG* is difficult to interpret.

The characteristics required of barley are similar for all malt distilleries. Since the barley malt is the sole source of carbohydrate the malt distiller selects grain with the greatest starch content. The extraction of the malt is designed to remove the maximum amount of soluble carbohydrate together with the soluble degradation products of proteins which provide an essential source of nutrients for yeast in subsequent fermentation. With the development of techniques to achieve the most efficient extraction of soluble constituents the uniformity in composition of the residual *MDG* between distilleries may be expected. Variations in the quality of *MDG* are unlikely to be due to differences in distillery practice.

The treatment of *MDG* after leaving the distillery may vary considerably with resultant variations in the quality of the material before it is fed to stock. The results of this study have shown that the method of transport is not an important factor in this respect and that losses are negligible. The changes in composition during storage of *MDG* are extensive particularly with respect to the soluble carbohydrate fraction which undergoes fermentation.

In the present study the effect of added salt has been studied by comparing the composition of salted and unsalted samples. The levels of *VFA* in the two treatments indicate that different types of fermentation have occurred. The increased level of butyric acid and reduction in propionic acid in the unsalted material suggests the existence of a clostridial type of fermentation. The addition of salt could cause suppression of clostridia by increasing the osmotic pressure.[11]

During relatively short periods of storage the losses are mainly due to fermentation through the loss of soluble constituents in the effluent and to gaseous losses of CO_2. Losses through spoilage are more important in storage periods of 3–6 months. The spoilage is due to the penetration of moulds and undesirable bacteria such as clostridia.

On a farm scale the loss of dry matter during long-term storage amounts to about 20% and the addition of salt does not appear to have any consistent effect. The increased losses with longer periods of storage may be largely accounted for by the increased spoilage.

The current practice of storing and feeding *MDG* in the wet state is a highly inefficient process because of these losses of nutrients. The soluble carbohydrate fraction of the dry matter which is most affected by fermentation is also the most readily digestible fraction when fed to the animal so that in

terms of digestible nutrients losses are even greater. Moreover, the penetration of moulds which cause spoilage can have deleterious effects when fed to the animal The removal of the spoilage is highly subjective and the possibility of inadvertantly feeding such material to stock is great.

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EVALUATION OF WHISKY DISTILLERY BY-PRODUCTS
II.*—Studies on the *in vitro* digestibility of malt distiller's grains

By G. A. EL HAG and T. B. MILLER

The *in vitro* digestibility technique has been used to determine the organic matter digestibility (*OMD*) of dried samples of malt distiller's grains (*MDG*).

The method gave highly reproducible results for *OMD* of fresh and ensiled samples when different batches of rumen liquor from hay-fed sheep were used. The results were consistently lower than values determined by *in vivo* digestibility trials.

The *OMD* values were increased by raising the calcium ion concentration of the digesta and by extraction of lipids from the *MDG* samples.

The addition of calcium had no effect on fat-extracted samples of *MDG*.

Introduction

In the previous paper[1] analytical data were given on the chemical composition of wet malt distiller's grains (*MDG*). These analyses showed the compositional differences between samples from various distilleries and the effect of storage of the material on the farm.

In general the samples of *MDG* were relatively high in cellulose, protein and lipid and low in soluble carbohydrate and minerals. These compositional characteristics are particularly important in determining the nutritive value of the material.

The relatively high lipid content is an important factor in the degradation of cellulose in the rumen. The earlier work of Kellner & Kholer[2] showed that when groundnut oil was added without emulsification to a basal ration the apparent digestibility fell. Half a century later similar findings were reported by Brooks *et al.*[3] who added corn oil to sheep rations. Levels of 5–7% of corn oil also lowered the digestibility of the cellulose fraction.[4,5]

Some studies have included the addition of agents which could reverse the effect of an increased lipid content on digestibility. Several authors[4,6,7] found that lucerne ash was effective in this respect. Later workers attempted to ascertain which of the major constituents of the ash was responsible for this improvement and the results showed that calcium was the principal factor. Grainger *et al.*[5,8] reversed the depression of the digestibility of organic matter and cellulose by adding calcium and concluded that the calcium requirements of the animal were increased by the addition of corn oil to the ration.

The rations studied by other workers had lipid added whereas *MDG* is an example of a food which contains 7–10% of lipid. As it has a high cellulose content and low soluble carbohydrate content it may be expected that the high lipid content would impair the digestibility of *MDG*. In estimating the nutritive value of the material it was therefore desirable to study the effect of the lipid fraction on digestibility.

The use of *in vivo* digestibility trials for this purpose was too time-consuming for effective study of all the parameters involved. Accordingly, an *in vitro* technique with rumen liquor from sheep was used. This procedure enabled simul-

* Part I: Preceding paper

taneous estimation of the digestibility of a relatively large number of samples treated in a variety of ways.

Experimental

Treatment of samples of *MDG*

The digestibility studies were conducted on samples of *MDG* which had been prepared in accordance with methods which were similar to those used in farm practice. On some farms the *MDG* is delivered at short intervals and fed to animals in a relatively fresh state before fermentative changes occur. Bulk collection and ensilage on other farms provides a somewhat variable product depending on the precautions which are taken. Common salt is frequently mixed with *MDG* before storage.

The samples of *MDG* were designated 'fresh', 'salted' and 'unsalted' according to the following treatments: 'fresh'—stored at −20° within 12 h of receipt from the distillery; 'salted'—ensiled in silos of 25 tons capacity and fed to stock over a period of six months. The samples were taken for digestibility studies after five months' ensilage. Salt was added at 1% of the wet weight of *MDG* during the filling of the silo; and 'unsalted'—ensiled without additives in 25 ton silos and sampled in the same manner as the salted material.

In the comparisons of *in vivo* digestibilities of fresh, salted and unsalted *MDG*, samples were prepared from a single distillery batch of *MDG*. The salted and unsalted portions were ensiled for 28 days and subsequently stored together with the fresh material at −20°. Cold storage of all samples before feeding was essential to avoid secondary fermentations which caused digestive disturbances in sheep.

The samples of *MDG* which were taken after 5 months and 28 days ensilage are referred to as 'long-term ensiled' and 'short-term ensiled' respectively.

In vitro digestibility estimation

The digestibility coefficients of organic matter (*OMD*) were estimated according to a routine procedure which is used in this laboratory for evaluating samples of dried herbage. The method is similar to that described by Alexander & McGowan.[9]

Samples of wet *MDG* were dried at 100° for 16 h and milled through a 0·8 mm sieve. Rumen liquor was withdrawn from sheep fed a maintenance diet of good quality hay. Of the dried milled material 0·5 g were incubated under anaerobic conditions with 50 ml rumen liquor which was diluted 4 times with McDougall's buffer. Additives were applied to the dried sample before addition of diluted rumen liquor. Anaerobic incubation was continued for 48 h after which the digest was acidified and incubated with pepsin for a further 48 h period. The procedure described by Alexander & McGowan was followed in detail with two modifications: (a) flushing with CO_2 after addition of pepsin was omitted and (b) orthophosphoric acid (50% by vol.) was used to adjust pH from the basic side.

In vivo digestibility trials

Six Suffolk cross Blackface wether lambs were used to determine the *in vivo* digestibility. The sheep were fed in digestibility crates which were built according to the design of Duthie.[10]

Fresh, salted and unsalted *MDG* were fed to each animal in accordance with a 3 × 3 latin square design. The *MDG* was fed *ad lib.* and supplemented with KCl, vitamin A and vitamin D. Fresh and unsalted samples were also supplemented with NaCl.

Results

Variations between batches of rumen liquor

The laboratory facilities for the *in vitro* estimation of *OMD* enabled up to 90 single estimations to be made simultaneously from one batch of rumen liquor. Quadruplicate determinations of digestibility were conducted on each sample of *MDG* or on each treatment sample.

To study the effect of different liquor batches on the *OMD* of individual samples, samples of long-term ensiled *MDG* (3 salted and 3 unsalted) were assayed using two out of six batches of rumen liquor which were withdrawn from sheep on six different days. The results are given in Table I.

There was close agreement between batches for the same material. The *OMD* values of salted samples of *MDG* were consistently greater than those of unsalted samples. The ratio of these two values (salted/unsalted) obtained on any particular day showed very little variation with different liquor batches.

Effect of adding calcium salts

The results in Table II show the effect of addition of calcium chloride and calcium acetate at two levels on the *OMD* of salted and unsalted long-term ensiled *MDG*.

TABLE I

***In vitro* digestibility (mean value with standard errors) of the organic matter, in salted and unsalted 'long-term ensiled' *MDG* samples using different batches of rumen liquor**

Rumen liquor batch	Date	Salted *MDG*		Unsalted *MDG*		Salted
		Sample	*OMD*, %	Sample	*OMD*, %	Unsalted
1	16.1.67	99	41·8 (0·07)	10	36·5 (0·15)	1·14
2	23.1.67	99	41·4 (0·31)	10	37·3 (0·37)	1·11
3	30.1.67	35	36·3 (0·49)	02	32·2 (0·65)	1·13
4	6.2.67	35	36·2 (0·67)	02	31·8 (0·42)	1·14
5	20.2.67	98	41·2 (0·31)	45	36·2 (0·07)	1·14
6	6.3.67	98	41·2 (0·68)	45	34·8 (0·34)	1·18

TABLE II

***In vitro* digestibility (mean values and standard errors) of the organic matter of long-term ensiled samples of *MDG*, both salted and unsalted, and the effect of adding calcium acetate and calcium chloride**

Additive*	Salted sample 99, *OMD* %	Unsalted sample 02, *OMD* %
Nil	41·2 (0·31)	32·5 (0·07)
0·1 m-mole Ca chloride	46·3 (0·42)	40·0 (0·68)
0·2 m-mole Ca chloride	45·0 (0·27)	38·8 (0·43)
0·1 m-mole Ca acetate	44·3 (0·36)	39·5 (0·08)
0·2 m-mole Ca acetate	42·6 (1·02)	39·4 (0·62)

* Amounts added per 0·5 g dried *MDG*

TABLE III

Comparison of *in vivo* and *in vitro* methods for the determination of the organic matter digestibility of fresh, salted and unsalted short term ensiled *MDG* and the effects of additional calcium

	Fresh, *OMD* %	Salted, *OMD* %	Unsalted, *OMD* %
In vivo	59·3 (0·16)	57·0 (0·44)	55·6 (0·64)
In vivo + Ca*	65·0	60·5	59·0
In vitro	52·0 (0·30)	48·2 (0·20)	47·4 (0·20)
In vitro + Ca**	58·4 (0·27)	55·4 (0·46)	55·1 (0·19)

* 4 g Ca as lactate per day
** 0·1 m-mole Ca chloride per 0·5 g dried *MDG*

Digestibility of the unsalted *MDG* was consistently lower than that of the salted material. The addition of calcium salts increased the digestibility of both types of distiller's grains. Increasing the calcium added from 0·1 to 0·2 m-mole gave no further improvement in digestibility. Results obtained with calcium chloride did not differ significantly from those obtained with calcium acetate, indicating that the nature of the anion associated with calcium is unimportant.

Comparison of *in vivo* and *in vitro* methods

The results in Table III represent the *in vivo OMD* values of fresh and short-term ensiled (salted and unsalted) *MDG* obtained with six sheep in a 3 × 3 latin square experiment together with values from two sheep in an unreplicated trial in which 4 g additional calcium as calcium lactate was fed per day. *In vitro* results from the same *MDG* samples are also included.

The *in vitro OMD* values were consistently lower than the corresponding *in vivo* values, but the results were in the same order by both methods, viz. fresh > salted > unsalted. The differences in digestibility between the fresh and stored samples were highly significant ($P < 0·01$) but there was no significant difference between the salted and unsalted samples. The *in vitro* values for salted and unsalted samples were appreciably higher than those of long-term ensiled *MDG* given in Tables I and II. This result may be attributed to the relatively short period of storage and freezing which reduced fermentation losses. The pattern of response to added calcium is similar by both methods.

Interaction of the lipid in *MDG* and added calcium salts

Comparative *in vitro* digestibility estimations were performed on samples of fresh and long-term ensiled salted and unsalted *MDG* samples and on the same samples after quantitative extraction of lipid with petroleum ether (b.p. 60°–80°). The effect of adding 0·1 m-mole $CaCl_2$ on digestibility was also studied, and the results are given in Table IV.

Removal of lipid resulted in an increase in the *OMD* of all three *MDG* samples. Similar increases in digestibility occurred when calcium chloride was added to the unextracted samples but a similar addition of calcium had no effect after the lipid was removed. These results demonstrate a definite interaction between lipids and calcium ions in the digesta.

Effect of varying the lipid content on the interaction with calcium salts

Admixtures of dried fresh *MDG* and the same sample extracted with 50% by vol. ethanol–benzene mixture were prepared to give material with lipid contents of 0, 1·5, 3·0, 4·5, 6·0 and 7·5%. *OMD* was estimated on each mixture with and without addition of 0·1 m-mole Ca/0·5 g sample.

The results which are illustrated in Fig. 1 show that the digestibility coefficients are inversely related to the concentration of lipid and the relationship is linear. Addition of 0·1 m-mole Ca gave a similar relationship between digestibility and percentage of lipid. The increases in digestibility associated with added calcium were greater at higher fat contents but all these raised values were appreciably below the digestibility of the fat-free *MDG*.

Discussion

The *in vitro* technique for the estimation of the digestibility coefficient of the organic matter fraction of *MDG* has given results which were consistently lower than those obtained *in vivo*. This result may be attributed to the use of rumen liquor from hay-fed sheep. It is possible that if the donor

TABLE IV

***In vitro* digestibility (mean values and standard errors) of the organic matter of *MDG* samples and the effect of removal of lipids and addition of calcium chloride**

Additive	Fresh *MDG*		Salted *MDG*		Unsalted *MDG*	
	Unextracted	Extracted	Unextracted	Extracted	Unextracted	Extracted
Nil	52·5 (0·17)	58·0 (0·56)	39·7 (0·36)	44·8 (0·77)	33·6 (0·44)	45·8 (0·66)
0·1 m-mole Ca chloride	54·8 (0·56)	57·9 (0·37)	47·0 (0·3)	45·3 (0·33)	43·0 (0·68)	45·4 (0·47)

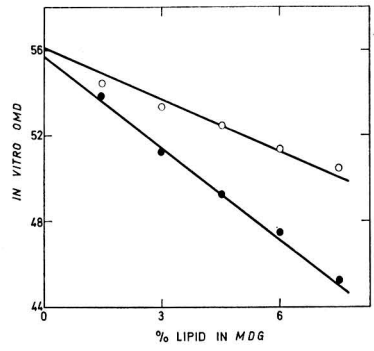

FIG. 1. *Effect of varying lipid levels on the* in vitro *organic matter digestibility* (OMD)

● Without added calcium
○ with addition of 0·1 m-mole per 0·5 g sample of *MDG*

sheep had been maintained on a diet of *MDG* a more appropriate microflora would have been established and the digestibility results would have agreed more closely with the *in vivo* values. Another criticism of the *in vitro* technique is the accumulation of products of digestion which are continuously absorbed from the gut of the intact animal, but remain in the test tube during the *in vitro* process. The procedure adopted in the present studies, however, has been developed for the evaluation of ground herbage samples, and conditions have been adjusted to attain close agreement with *in vivo* values.

The absence of close agreement between the *in vivo* and *in vitro* values has not invalidated the application of the *in vitro* technique in the present investigation. The relative differences between different samples of *MDG* and between batches of rumen liquor have been consistent. The technique has proved most useful for study of a number of parameters simultaneously and elucidation of the effects of the presence of lipids on digestibility.

The reduction in digestibility of *MDG* with ensilage may be attributed to the degradation of soluble carbohydrate through fermentation. The volatile fatty acids produced in fermentation and lost in drying could also account for part of the reduction. The period of storage is an important factor since *MDG* samples subjected to long-term storage showed a greater reduction in digestibility than material stored for about 4 weeks. The slight difference between salted and unsalted samples is not due to the presence of salt *per se* since addition of NaCl to unsalted *MDG* had no effect on digestibility (El Hag, G. A., & Miller, T. B., unpublished results). The increased degradation of carbohydrate is probably the main factor. With the salted samples, degradation is less extensive and the material is more digestible. These results are in accord with compositional data reported by Miller.[1]

The increase in digestibility of organic matter of *MDG* by raising the concentration of calcium ions in the digesta or from removal of the fat fraction, and the absence of any effect on digestibility when calcium salts were added to fat-free *MDG*, demonstrate the interaction between the lipid and calcium. Moreover, the nature of the anion associated with the calcium is unimportant though only chloride and acetate were investigated. Grainger *et al.*[5] found that the presence of potassium ions enhanced the effect of fat but the presence of sodium salts in the present study did not demonstrate this effect. This apparently conflicting result may be attributed to the use of an *in vitro* technique in the present work whereas Grainger *et al.* used digestibility trials with sheep.

The linear relationship obtained between level of lipid and depression in digestibility, when the lipid concentration was increased from 0 to 7·5%, does not agree with the results of *in vivo* studies reported in the literature. Buysse[11] concluded that lipid concentrations below 5% did not influence digestibility. It is possible that the *in vitro* technique affords greater precision in this respect and that compensatory factors in the animal may mask any effect of lipid at low concentrations.

Grainger *et al.*[5] have attributed the effect of lipid on digestibility to the bacteriostatic action of long-chain fatty acids on the cellulolytic bacteria of the rumen. The calcium ions can counteract the effect of the fatty acids by precipitation of insoluble calcium soaps. The present findings are in accord with these hypotheses though subsequent studies in this laboratory on the differential effects of other alkaline earth metals indicate that the mode of action of lipid and calcium is more complex.

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PERSISTENCE AND EFFECTIVENESS OF THIONAZIN AGAINST POTATO APHIDS ON THREE SOILS IN SOUTHERN ENGLAND

By B. F. PAIN and R. F. SKRENTNY

Degradation curves were determined for thionazin applied to the soil in potato plots at three sites. Thionazin persisted longer in an acid, sandy soil than in an alkaline, clay soil, and residues were detected in the sandy soil a year after application. Results of residue analyses were confirmed by determining the kills of aphids (*Myzus persicae* Sulz.) 'sleeved' on to potato plants. Natural infestation by potato aphids was effectively decreased only on plots treated with thionazin at 40 lb/acre (44·84 kg/ha) on the acid sandy soil.

Introduction

Unlike some other methods of pest control, soil-applied pesticides may not come into contact with the pest directly, but only after water dispersal and diffusion into and through the soil and, with systemics, uptake through the root systems to the upper part of the plants. The efficacy of a soil-applied organophosphorus pesticide depends on many factors including its persistence as a toxic agent in the soil, which for present day chemicals is relatively short compared with that of some organochlorine compounds.[1] An understanding of the factors which influence the persistence of such pesticides between the time of application and contact of the target organism is important. In recent years it has been shown that persistence and effectiveness of soil-applied pesticides differ according to soil type.[2,3] Thus it is important to know the persistence and effectiveness of each pesticide on a range of contrasting agricultural soils to determine the appropriate dosage level needed for control. The accumulation of this type of information is not only important in determining seasonal control recommendations, but also as background information for the study of long-term biological effects and pesticide-residue accumulations in soils, and in the environment as a whole.

This investigation was initiated during the growing season of 1967 with thionazin (Zinophos^R, *O,O*-diethyl *O*-2-pyrazinyl phosphorothioate) since it is one of a group of promising soil-applied organophosphorus pesticides used to control certain soil insects and nematodes, as well as being a systemic aphicide. Three British agricultural soils, representing a range of pH values, clay content, and sand content, were selected for this study in an attempt to determine the effectiveness of thionazin as a field-applied systemic pesticide against potato aphids. Analysis of thionazin residues in soil and plants was combined with this aphid study to aid in the interpretation of the biological results and also to determine the persistence of thionazin during the growing season in these contrasting soil types.

Experimental

The experiments consisted of blocks of six plots, each 12 yd (10·96 m) × 6 yd (5·48 m), with a 1 yd (0·91 m) 'buffer-strip' between each, laid out in Church Field, Silwood Park, in Partridge Field, Weed Research Organisation, Begbroke, Oxon. and in High Field, Grassland Research Institute, Hurley, Berks. The plots were planted with potatoes in April, 1967. Samples were taken from each plot and analysed to ensure that each was free from interfering pesticides. Shortly after planting, plots were treated with thionazin at two dosage rates; high dosage was equivalent to 40 lb active ingredient per acre (44·84 kg/ha); low dosage to 10 lb active ingredient per acre (11·21 kg/ha). Each treated plot was duplicated and two untreated plots were included as controls. Plots to be treated with pesticide were marked off by strings into 1 yd (0·91 m) squares and thionazin was applied as a granular preparation, containing 10% active ingredient, by sifting the appropriate amount on to each square. After application, the granules were lightly raked into the top inch of soil.

At each sampling date, 18 random sub-samples were taken from each plot to a depth of 6 in (152·4 mm) with an auger 1 in (25·4 mm) in diameter. To avoid 'edge-effects', the outer rows were excluded from the sampling scheme. Sub-samples from each plot were pooled and thoroughly mixed. Soil moisture was determined on a 25 g aliquot of each sample to enable the results of residue analysis to be expressed on a dry weight basis. A further 100 g were weighed into a glass jar, and 100 ml of a solvent mixture consisting of 8 parts benzene, 1 part hexane and 1 part acetone, were added to it. The solvent–soil mixture was homogenised with a M.S.E. High Speed Homogeniser for 15 minutes. Any solvent lost by evaporation during this process was made up, and the jar was tightly capped and allowed to stand for at least eight hours. The supernatent solvent was decanted off into a screw-cap jar containing anhydrous sodium sulphate to remove any remaining water, and was stored in a refrigerator at 1–2° until analysed. Extraction efficiency, using this method, and solvent mixture, was between 95 and 101%.

Mature potato leaves were collected from each plot 6 and 10 weeks after application of pesticide. Leaves from duplicate plots were mixed, cut up with scissors, and minced. Residues were extracted by a method similar to that described for soil, 50 g of minced leaves being homogenised for 5 min with 200 ml of the solvent mixture. When residues were small it was necessary to concentrate the extract and to use a clean-up technique based on the sweep codistillation method.[4]

Thionazin residues were estimated on a Varian Aerograph 1200 gas–liquid chromatogram equipped with an electron-capture detector containing a tritium source and a stainless steel column (1 metre × 3·17 mm o.d.) containing Chromosorb W (60 to 80 mesh) coated with silicone grease (5% by wt.). Detector base temperature was maintained at 280° (equivalent to foil temperature of 170°), column at 160° and injector at 170°. The carrier gas (N_2) flow was maintained at 30 ml/min. Under these conditions the retention time of thionazin was 2 min.

Soil organic matter was determined by the wet oxidation method and mechanical analysis by the pipette method.[5] pH was determined on a 1 : 2·5 soil–water suspension with a pH meter fitted with glass electrodes.

Sleeves made of organdie were used to cage a known number of laboratory-reared *Myzus persicae* (Sulz.) to potato plants at each site. Usually three sleeves containing 20 apterae between the fourth-instar and young adult stages were put on each plot on young, medium and old leaves chosen at random from the central rows of each plot. Care was taken to remove existing aphids, predators and parasites from the leaves to be sleeved. Sleeves were left on in the field for 8 days and then removed, and the number of dead and live adult *M. persicae* and their progeny were counted and recorded. Weekly assessments of aphids naturally infesting potato crops were made to determine their prevalence in relation to treatments. Four stems were selected at random from the central rows of each plot, cut off at the base and put singly into labelled plastic bags and taken to the laboratory where leaves were stripped off and examined for aphids.

Results

Thionazin residues were determined on soil extracts from samples taken from each plot at intervals up to 12 weeks after the initial application. Residues expressed as parts per million dry weight of soil are shown graphically in Figs 1 and 2, each point representing the mean result, as determined by g.l.c. analysis, from two duplicate plots. Initial levels were not determined by analysis, but calculated on the basis of dose applied and soil density, and were approximately 20 ppm in the 'high-dosage' plots and 5 ppm in the 'low-dosage' plots.*

After clean-up of the potato leaf extract as described above, interfering peaks on the chromatogram still tended to mask the presence of very small amounts of thionazin. The limit of detection of thionazin in plant extract was, therefore, 0·05 ppm, and small unresolved peaks, not accurately measureable because of interfering peaks, were recorded as a trace amount. Six weeks after application, a trace of thionazin was still detectable in plants in 'low-dosage' plots at Church Field and in all plots in Partridge Field, while 2·6 ppm was recorded in samples from 'high-dosage' plots in Church Field. No residues were detected in leaf extracts from High Field at this time. Ten weeks after application, a trace was still present in the 'high-dosage' Church Field plots, but no thionazin was detected in samples from other sites.

Results of g.l.c. residue analyses were confirmed by the tests with aphids sleeved on to the plants at intervals during the season. Church Field and High Field provided the greatest contrast in terms of soil types (Table I) and of thionazin residues. Aphid tests began at these sites in June. Mean numbers of naturally occurring aphids collected from each potato stem were plotted against time after application of thionazin (Figs 3 and 4). All aphids were not identified, but

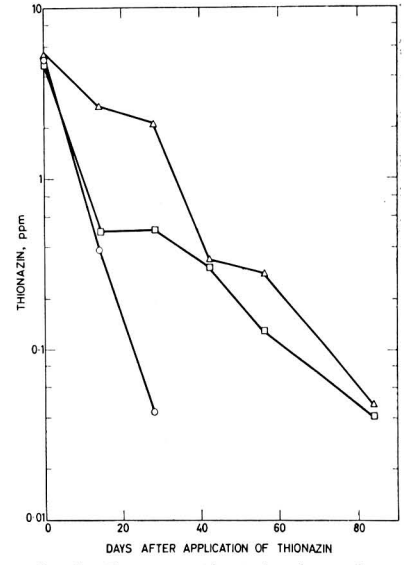

FIG. 1. *Thionazin residues in low-dosage plots*
○——○ High Field; □——□ Partridge Field; △——△ Church Field

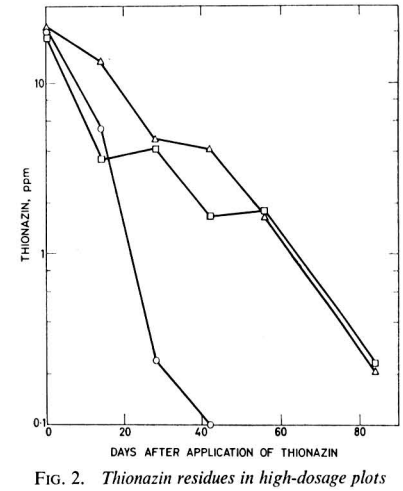

FIG. 2. *Thionazin residues in high-dosage plots*
○——○ High Field; □——□ Partridge Field; △——△ Church Field

Myzus persicae (Sulz.) and *Macrosiphon euphorbiae* (Thos) were predominant. Aphids arrived on the plants in June, reached peak numbers in early July and began to decline in August as the crop matured. The 'high-dosage' treatment in Church Field effectively decreased the infestation during this period, and the 'low-dosage' treatment gave some control. Numbers on treated plots in High Field did not differ from those on untreated controls. The mean percentage mortalities of *M. persicae* sleeved on to potato plants in treated plots, were corrected for control mortality and results for both sites are shown in Fig. 5.

* Recent results obtained by gas–liquid chromatography analysis of soil samples from the Church Field low dosage broadcast plots (10 lb/acre) taken one year after application of thionazin indicate a residue level of 0·025 ppm in the top 0–6 inch layer and 0·028 ppm in the 6–12 inch layer. No thionazin residue was found in the 12–18 inch layer nor was any detectable amount of thionazin present in any soil layers at High Field. An additional check for the presence of thionazin in these soil extracts was made by use of thin-layer chromatography in which silica gel plates were developed in hexane–chloroform–methyl alcohol (7:2:1) followed by a spray of 0·5% palladium chloride and then 5 N sodium hydroxide.

TABLE I
Soil characteristics

Site	Field capacity	Bulk density	% Organic matter	pH	Mechanical analyses			
					Silt	Clay	Fine sand	Coarse sand
Church Field, Silwood	19·8	1·38	2·4	5·4	15·3	5·1	49·3	29·5
Partridge Field, W.R.O.	21·0	1·31	1·7	6·4	15·0	12·9	22·5	49·9
High Field II, G.R.I.	22·3	1·29	2·2	7·3	12·4	24·7	27·9	32·9

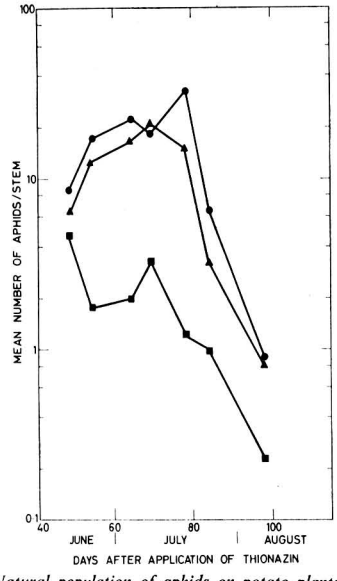

FIG. 3. *Natural population of aphids on potato plants in Church Field plots*
●—● Control; ▲—▲ Low Dosage; ■—■ High Dosage

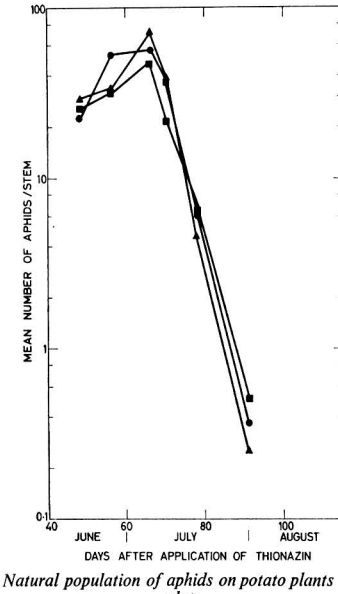

FIG. 4. *Natural population of aphids on potato plants in High Field plots*
●—● Control; ▲—▲ Low Dosage; ■—■ High Dosage

Discussion

The results demonstrate that the rate of disappearance of thionazin varies considerably on different agricultural soil types. The acid sandy soil of Church Field provided conditions best suited for the long-term persistence and systemic efficiency of thionazin. Detectable amounts of thionazin remained up to one year in Church Field soil whereas in the higher-clay alkaline soil of High Field no residues were detected after this period. These results correspond closely with those of Getzin & Rosefield[2] for similar soils.

Way & Scopes[6] obtained evidence, using a Collembola bioassay technique, which indicated that on a similar site and soil at Silwood Park a single application of thionazin was still detectable as a toxic residue the next growing season. Scopes & Lichtenstein[7] have demonstrated that Collembola are exceedingly sensitive to very small residues of organophosphorus pesticides less than one microgramme in amount. Under the favourable conditions for thionazin persistence in Church Field soil, sufficient toxicant remained over winter to be detected by Collembola bioassay in the next growing season. The formation of a stable non-detected toxic metabolite of thionazin cannot be excluded but other research workers using a similar sandy soil and radioactively labelled, thionazin were unable to detect any metabolites.[2,8]

Since the distance between Church Field and High Field is less than 20 miles (32 km), climate would not seem to be the major factor influencing the difference in persistence of thionazin. The faster degradation and lower systemic efficiency of thionazin in High Field may be due, in part, to a more rapid hydrolysis in this alkaline soil coupled with an irreversible sorption on the clay particles. Getzin & Rosefield[2], working with labelled thionazin, stated that in clay loams a rapid increase in soil-bound radioactivity was found two weeks after soil treatment and continued to increase slowly over the 24 weeks of their study. Several workers in the U.S.A.[2,9,10] have suggested that microbiological degradation is an important factor affecting the persistence of organophosphorus pesticides. Getzin & Rosefield[11] have shown that certain unidentified, heat-labile substances present in the soil may also play a part in degrading pesticides.

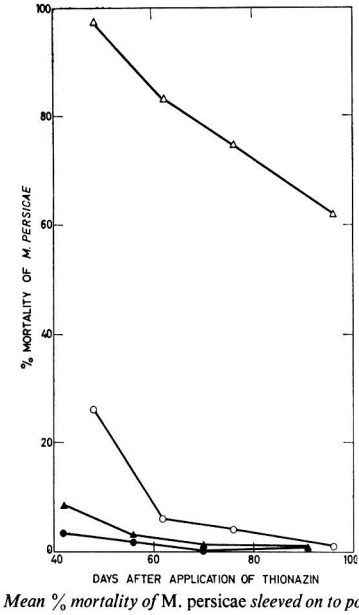

FIG. 5. *Mean % mortality of* M. persicae *sleeved on to potato plants*
Church Field: △—△ High Dosage ○—○ Low Dosage High Field: ▲—▲ High Dosage ●—● Low Dosage

'High-dosage' treatments at High Field killed less than 10% of *M. persicae* caged on to potato plants 42 days after application of thionazin. At the same time, 30% mortality was obtained in the 'low-dosage' plots at Church Field and nearly 100% in the 'high-dosage' plots. These results are closely correlated with results of g.l.c. analyses of thionazin residues in soil and plants. However, at Church Field many aphids were still being killed 13 weeks after application, although g.l.c. analysis indicated exceedingly low levels of thionazin in soil and plants; this emphasises the need for biological assay as well as residue analysis in this type of investigation. Thionazin treatments did not decrease the number of naturally occurring aphids at the High Field site at any time during the season. At Church Field early infestation was reduced on plants in all treated plots but subsequently the low dosage was not effective in controlling aphids; the high dosage gave effective protection against aphids throughout the season.

These results indicate that agricultural soil type should be taken into account when appropriate dosage levels are suggested for control of pest organisms. Manufacturers of soil-applied pesticides often make only a general recommendation for the application of their product to all soil types. For example, a soil broadcast treatment of thionazin from 6 to 16 lb a.i./acre (6·7 to 17·8 kg/ha) is recommended[12] for aphid and mite control on ornamental plants, without any reference to the possible effects of various soil types on the efficiency of this application. In this study, of the two dosage rates used only the high rate of 40 lb/acre was effective as a systemic control against aphids, and then only at one of the two sites considered. This rate of application is unrealistic in terms of farm practice and was applied here for experimental contrast. Broadcast application of thionazin was used to facilitate sampling for analytical work, whereas practical control of aphids would, no doubt, be best with an in-row or band application requiring less chemical per acre.

Acknowledgments

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DIFFUSION OF ORGANOPHOSPHORUS INSECTICIDES IN SOILS

By I. J. GRAHAM-BRYCE

Diffusion of disulfoton and dimethoate in a silt loam soil was studied over a range of concentrations and moisture contents. Apparent diffusion coefficients were calculated from the distribution of insecticide in a column of soil after diffusion from one half of the column to the other for a known time. The distribution was determined by slicing the column into narrow sections using a specially constructed diffusion cylinder. Diffusion coefficients varied little with concentration for both insecticides, but increased rapidly with increasing moisture content for dimethoate from $3{\cdot}31\times10^{-8}$ cm²/sec at 10% volumetric moisture content to $1{\cdot}41\times10^{-6}$ cm²/sec at 43% moisture content. In contrast, for disulfoton which is more volatile, less soluble and more strongly sorbed than dimethoate, diffusion coefficients were smaller ($2{\cdot}83\times10^{-8}$ cm²/sec at 41% moisture content) but did not change much as the soil became drier ($2{\cdot}74\times10^{-8}$ cm²/sec at 8% moisture content). The influence of partition between solid, solution and vapour phases in the soil and of the geometry of the pathway through the soil pores on the apparent diffusion coefficient is discussed. The likely behaviour of other pesticides is considered in the light of these results.

Introduction

A knowledge of the speed at which pesticides diffuse through soil is necessary to calculate distributions after they are applied to soil and to estimate amounts that can move to plant roots. Measurements of diffusion rates can also give indirect information about interactions between pesticides and soil solids. Diffusion of fumigants such as ethylene dibromide has been studied in detail[1] and measurements for 2,4-D in saturated soils have been reported recently,[2] but other pesticides do not seem to have been studied quantitatively.

Insecticides diffuse more slowly in soil than in air or free solution because the pathway through the pores is restricted and tortuous and because part of the chemical may be retarded by sorption on the solids. The exact geometry of the pathway depends on the nature of the pore space formed by the particles in a given soil and on the moisture content. The way these geometric factors influence diffusion of a pesticide depends on its solubility and volatility, which govern the relative amounts moving in the soil solution or as vapour in the soil air space. According to Fick's law, the amount of insecticide diffusing/second through unit area of soil at any point is the product of the gradient of concentration, and the apparent diffusion coefficient whose value is determined by the geometric and partition factors just discussed. Measuring diffusion coefficients has the advantages over less precise estimates of the apparent distances which pesticides diffuse in that the values can be used to predict distributions in systems different from those used in measurement; and also that the contribution of different factors to the rate of diffusion can be calculated in detail. This paper reports determinations of diffusion coefficients of the systemic insecticides dimethoate (dimethyl *S*-(*N*-methylcarbamoylmethyl)phosphorothiolothionate) and disulfoton (diethyl *S*-[2-(ethylthio)ethyl]phosphorothiolothionate) in soil of various moisture contents. These insecticides were chosen because they have contrasting physical properties and affinities for soil. Dimethoate is soluble in water to about 3% by wt. and has a vapour pressure of $8{\cdot}5\times10^{-6}$ mm Hg at 20°, whereas disulfoton is much less soluble (about 15 ppm at 20°) and is more volatile (v.p. $1{\cdot}8\times10^{-4}$ mm Hg at 20°; vapour pressures and solubilities from technical data given by manufacturers, and from Lord & Burt[3]).

To help analyse the contribution of different factors to the values of diffusion coefficients, sorption isotherms for these insecticides on soil were also determined.

Theoretical

The determination of diffusion coefficients for insecticides has been discussed briefly by the author;[4] fuller details are now given.

A satisfactory method for determining diffusion coefficients must allow for the possible dependence on concentration. The appropriate forms of Fick's law of diffusion are:

$$F = -D\frac{\partial C}{\partial x}$$

where

F = flux of diffusing insecticide in the x direction (g/cm²/sec)
C = concentration of insecticide in whole soil (g/ml)
D = apparent diffusion coefficient (cm²/sec)

and

$$\frac{\partial C}{\partial t} = \frac{\partial}{\partial x}\left(D\frac{\partial C}{\partial x}\right) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

For the boundary conditions at $t=0$, $C=C_0$ for $x>0$ and $C=0$ for $x>0$ and for all values of t at $x=\pm\infty\,\partial C/\partial x=0$. D can be obtained from Equation (1) using the method of Matano.[6] Applying the Boltzmann transformation $\lambda=x/(t)^{\frac{1}{2}}$, gives the following solutions for these boundary conditions:

$$\text{for } x<0,\ D = -\frac{1}{2t}\frac{\mathrm{d}x}{\mathrm{d}(C/C_0)}\int_0^{C/C_0} x\ \mathrm{d}(C/C_0) \quad \ldots\ldots\ldots\ldots \quad (2)$$

$$\text{for } x>0,\ D = \frac{1}{2t}\frac{\mathrm{d}x}{\mathrm{d}(C/C_0)}\int_{C/C_0}^{1} x\ \mathrm{d}(C/C_0) \quad \ldots\ldots\ldots\ldots \quad (3)$$

Hence, when the distribution of insecticide resulting from diffusion for a known time t in a system corresponding to the boundary conditions is determined, D can be evaluated at different values of C/C_0 by measuring $\mathrm{d}x/\mathrm{d}(C/C_0)$ and $\int x\ \mathrm{d}(C/C_0)$ graphically on the x–C/C_0 curve. A mean value, $\bar{D}$, for the concentration range studied can be obtained from a plot of D against C/C_0. This method was recently applied by Phillips & Brown[6] for measuring ionic counter-diffusion in soil.

Insecticide may move through the gaseous and liquid phases in soil and sorbed insecticide may move along surfaces or in the solid phase. However, the pesticide associated with the solid almost certainly diffuses much more slowly than in the pores and therefore makes a negligible contribution to the total flux, even when most of the pesticide occurs on the solid because of strong sorption. Sorption isotherms for pesticides are usually approximately linear (for examples see literature cited by Graham-Bryce[7]) and if Henry's law holds for the insecticide vapour, partition between solid, solution and vapour should be independent of concentration. D should then also be independent of concentration, assuming that equilibration between the phases is rapid compared with the change in concentration resulting from diffusion and neglecting the minor influence of concentration on diffusion coefficients in free air and free solution.

The relative contributions of the liquid and vapour pathways to the value of the diffusion coefficient will depend on the volatility and solubility of the pesticide. Volatile but sparingly soluble compounds may diffuse almost entirely through the vapour phase whereas this pathway would be insignificant for soluble, involatile materials. With such extreme compounds that diffuse effectively through only one pathway:

$$D\frac{dC}{dx} = D_Z V_Z f_Z \frac{dC_Z}{dx}$$

where the subscript Z refers to either the liquid (L) or gaseous (G) phase, V is the fraction of the soil occupied by the phase referred to, f is a geometric factor which allows for the tortuosity of the diffusion path through the soil pores, D_Z is the diffusion coefficient in free solution or free air and C_Z is the concentration/unit volume of the phase. Hence:

$$D = D_Z V_Z f_Z \frac{dC_Z}{dC}$$

If Henry's law holds and the adsorption isotherm is linear, dC_Z/dC is a constant so that:

$$D = D_Z V_Z f_Z \frac{C_2}{C} \quad \cdots\cdots\cdots\cdots\cdots\cdots (4)$$

Nye[8] developed similar equations for ionic diffusion in soils.

Experimental

Soil

Soil from the top 15 cm of the plot on Broadbalk field at Rothamsted Experimental Station which has been receiving farmyard manure annually since 1843 was air-dried and sieved (< 2 mm) before use. This soil is a silt loam and the properties of the sample used were clay 18%; cation exchange capacity 19·8 mequiv./100 g, pH (1:2·5 suspension in water) 7·8; organic carbon 2·7%.

Determination of diffusion coefficients

The experimental system consists of a cylinder of soil mixed uniformly with insecticide at a concentration C_0 joined to a similar cylinder initially free from the insecticide. After time t, the insecticide has diffused so that it is distributed as shown in Fig. 1. This system corresponds to the boundary conditions of the Matano method provided that t is short enough for the assumption that x extends infinitely in both directions to be valid. The concentration along the cylinder is found by slicing it into narrow sections and measuring the insecticide in each. The values of $dx/d(C/C_0)$ and $\int x\, d(C/C_0)$ in Equations (2) and (3) correspond to the tangents and areas indicated in Fig. 1 where examples are given for $C/C_0 = 0{\cdot}2$ and $0{\cdot}8$.

Fig. 2 shows the apparatus used to obtain the experimental system. The two halves of a brass tube (5·5 cm long × 3 cm dia.) are joined by a brass coupling ring, which runs on a thread machined on the outside of the tube. A small rabbet

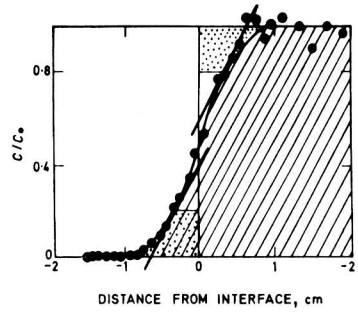

FIG. 1. *Experimental system for measuring diffusion coefficients*

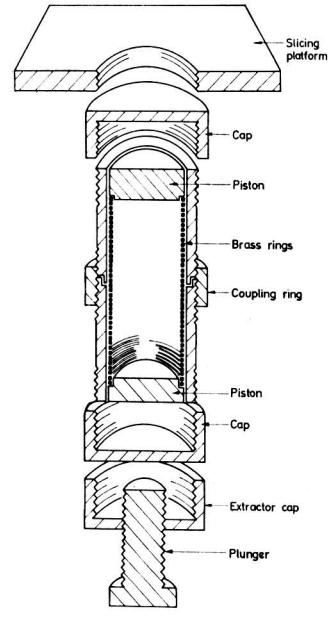

FIG. 2. *Diffusion cylinder*

ensures that the two halves fit tightly together. A cap with washer closes each end of the tube. Fitting exactly inside this outer tube is a second cylinder composed of 40 brass rings (axial thickness 1 mm, annular thickness 1 mm) machined flat to fit flush with each other. At each end of the inner cylinder is a brass piston, which also fits exactly inside the outer cylinder. A 1 mm rabbet in the pistons fits into the inner cylinder when the caps are tightened and presses the soil towards the centre.

Soil to be used in diffusion experiments is first equilibrated with the appropriate solutions by shaking on an end-over-end shaker. For disulfoton, which is appreciably sorbed, about 25 g portions of soil were shaken eight times with 100 ml of nearly saturated disulfoton solution in 0·01 M-$CaCl_2$. The suspensions were centrifuged and the supernatant fluid was discarded between shakings. For dimethoate, shaking twice with 0·1% solutions was sufficient and gave soil concentrations of the same order as those obtained with disulfoton. With each insecticide a similar portion of soil was shaken under identical conditions with 0·01 M-$CaCl_2$ only, to provide soil for the half of the cylinder free from insecticide. After the final equilibration, the suspensions were filtered using a Buchner funnel and suction was applied for a further 30 min after all supernatant fluid had been removed. This gave a soil with a reproducible moisture content, which was passed damp through a 2 mm sieve to produce small aggregates. When drier soil was required, these aggregates were dried for an appropriate time with constant stirring under an infra-red lamp using standardised conditions. For measurements in soil wetter than the aggregates, the required additional portions of the final equilibrating solutions were added dropwise with a pipette to layers of the prepared soil during packing into the diffusion cylinders.

For packing, the cylinders were separated into the two halves, and the pistons reversed so that the rabbet would not compress the soil. Successive weighed portions of prepared soil, sufficient to occupy 4 rings of the inner cylinder, were carefully tamped down into the appropriate volume so that there was a uniform bulk density of 1·25 g dry soil/ml through the column. When full, the half-cylinders were sealed and left for at least 24 h to allow moisture conditions to equilibrate in the aggregates. The treated and untreated halves of each cylinder were then joined tightly using the coupling ring, the pistons reversed and the caps screwed tight so that the slight compression produced by the rabbets ensured contact between the two halves of the soil column. The cylinders were kept at 20° in constant temperature rooms for a suitable period while diffusion took place. One end cap was then replaced by an extractor cap fitted with a threaded plunger (1 mm pitch) and the other end cap replaced by a flat slicing platform which was screwed down flush with the top of the diffusion cylinder. The cylinder was held vertically in a clamp and the inner cylinder extruded slowly upwards from the outer tube by turning the plunger. As each ring emerged, it was removed and the soil sliced off using a single hollow-ground razor. The soil sections were dried with sodium sulphate and the insecticide was extracted for analysis by gas–liquid chromatography. Disulfoton was extracted as described for measuring adsorption isotherms (see below) and dimethoate was extracted using 10 ml acetone/g soil. From a plot of the distribution of insecticide along the soil column, diffusion coefficients were calculated using the appropriate equations. Each experiment was replicated three times. Moisture contents were checked gravimetrically for sample sections along each cylinder.

Adsorption isotherms

The determination of sorption isotherms for disulfoton has been described previously.[7] 2 g portions of soil were equilibrated for 16 h with 100 ml disulfoton solutions having a range of initial concentrations 2–14 ppm in 0·01 M-$CaCl_2$. After centrifuging, the equilibrium concentration of disulfoton in solution was found by extracting into hexane and analysis by gas–liquid chromatography. Disulfoton sorbed was determined by extracting the soil, after decanting the supernatant solution and drying with anhydrous sodium sulphate, using 15–30 ml of solvent containing 3 parts acetone to 2 parts hexane. Acetone was removed from the mixed extract by shaking with 2% sodium sulphate solution and disulfoton in the remaining hexane was determined by gas–liquid chromatography.

Much less dimethoate was sorbed than disulfoton, so that 10 g portions of soil were equilibrated with 20 ml dimethoate solutions (10 ppm–2%) in 0·01 M-$CaCl_2$. Even with this large soil/solution ratio, gas–liquid chromatography had too large an experimental error to measure reliably the decrease in solution concentration during equilibration. Also, it was difficult to determine amounts sorbed by extracting the soil, because the solution retained by the soil after centrifuging contained very much more dimethoate than was sorbed. Sorption was therefore studied using ^{32}P-labelled dimethoate and amounts on the solid calculated from the decrease in solution concentration only, assuming that all material lost from solution was taken up by the solid. Radioactivity before and after equilibration was measured by counting 10 ml portions of the aqueous solution using a liquid Geiger-Müller counter and a Panax GX9 autoscaler. For adsorption isotherms, amounts taken up by the soil were plotted against equilibrium solution concentrations.

Gas–liquid chromatography

An Aerograph 1520 gas chromatograph with a thermionic phosphorus detector was used. Operating temperatures were injector 210°, column 195° and detector 215°. The stainless-steel columns were 3 mm × 75 cm for dimethoate and 3 mm × 150 cm for disulfoton and were packed with 5% SE30 on 60/80 Chromosorb W. The instrument was calibrated each day with standard solutions, and the calibration was checked at intervals during a run. Each extract was injected at least twice.

Results and Discussion

The equations for calculating diffusion coefficients were derived assuming that C/C_0 was a function of $x/(t)^{\frac{1}{2}}$, i.e. that changes in the distribution of insecticide in the cylinders with time were caused only by normal diffusion so that the distance moved by any value of the relative concentration (C/C_0) was proportional to $(t)^{\frac{1}{2}}$. The validity of this assumption must be tested, especially because organophosphorus insecticides are transformed chemically and microbially in soil. Evidence of decomposition was obtained by extracting test samples of soil. The amounts extracted decreased to about 70% of the initial value after one week with disulfoton and to about 60% with dimethoate. Although diffusion experiments were usually completed in 3–5 days therefore, the distribution of insecticide could be significantly affected by processes other than diffusion. If the assumption that C/C_0 is a function of $x/(t)^{\frac{1}{2}}$ is valid, a plot of x, the distance moved by a given value

of C/C_0 against $(t)^{\frac{1}{2}}$ should be a straight line passing through the origin. Fig. 3 shows results of experiments to test this. For this purpose C_0 was taken as the concentration determined in sections of the treated soil sufficiently far from the boundary to be unaffected by diffusion.

With both disulfoton and dimethoate, agreement with theory is reasonable, showing that any decomposition of the parent molecules which occurred did not affect the shape of the distribution curve much. This would be the case if the transformations were first order with respect to insecticide concentration.

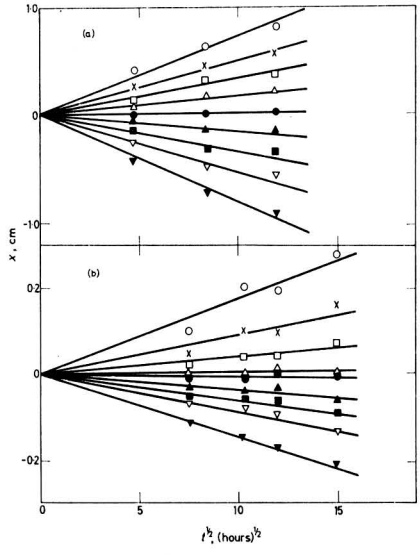

FIG. 3. *Test of validity of diffusion equations*
(a) Dimethoate; (b) disulfoton
C/C_0: = 0·9 (○); 0·8 (×); 0·7 (□); 0·6 (△); 0·5 (●); 0·4 (▲); 0·3 (■); 0·2 (▽); 0·1 (▼)

Table I gives results of diffusion measurements at different soil moisture contents. Because of the limited movement and the few points available, and because of the experimental errors associated with gas–liquid chromatographic analysis of the soil extracts, the exact shape of the disulfoton distribution curve was often difficult to draw at large or small values of C/C_0 where the curvature is steep. Also, with both insecticides, the slopes represented by $dx/d(C/C_0)$ are more difficult to measure accurately where the curvature is steep. The method is therefore least reliable at extreme values of C/C_0 and results in Table I are confined to values of C/C_0 from 0·2 to 0·8.

Concentration had little effect on the diffusion coefficient except that values tended to be somewhat smaller at intermediate concentrations. However, this effect was not consistent and seemed independent of the value of C_0, so it may be attributed to experimental error. Fig. 4 shows that the adsorption isotherm for disulfoton is linear and although the dimethoate isotherm is better described by the Freundlich equation, the curvature is small so that a straight line is a good approximation over the concentration range studied. Unless C_G/C varied markedly with concentration, therefore, only a small effect of concentration on D would be expected.

From the results in Table I, average diffusion coefficients, $\bar{D}$, can be obtained for the concentration range studied. Fig. 5 shows how soil moisture content influences these average values. Moisture content has little effect on the diffusion of disulfoton, but with dimethoate the effect is considerable, indicating the very much greater importance of the solution pathway in this case.

Consideration of the relative importance of liquid and vapour diffusion for these two compounds allows some further interpretation of these results. As already discussed, vapour diffusion can be neglected for pesticides of small volatility

TABLE I

Apparent diffusion coefficients ($D \times 10^7$ cm^2/sec) for dimethoate and disulfoton at different concentrations in Broadbalk soil over a range of moisture contents

Insecticide	Volumetric moisture content, %	C_0, mg/ml whole soil	C/C_0						
			0·2	0·3	0·4	0·5	0·6	0·7	0·8
Dimethoate	10·4	0·425	0·32	0·27	0·29	0·31	0·34	0·37	0·50
	17·4	0·396	1·31	1·20	1·02	0·96	1·13	1·22	1·32
	23·4	0·350	2·74	2·45	2·26	1·94	1·82	1·91	2·15
	31·6	0·430	5·94	4·63	4·34	4·10	4·29	4·68	4·84
	32·8	0·370	6·11	6·16	5·75	5·68	5·47	5·54	4·80
	35·6	0·421	9·42	9·65	9·70	9·44	9·00	8·56	8·82
	42·9	0·535	14·05	13·27	12·59	13·08	14·03	16·00	15·91
Disulfoton	7·7	0·298	0·35	0·24	0·23	0·23	0·27	0·31	0·37
	16·4	0·246	0·23	0·22	0·22	0·23	0·26	0·29	0·30
	21·6	0·096*	0·20	0·18	0·17	0·19	0·18	0·24	0·27
	23·0	0·256	0·20	0·20	0·18	0·18	0·18	0·22	0·28
	30·1	0·271	0·19	0·15	0·14	0·16	0·18	0·34	0·47
	31·9	0·226	0·20	0·16	0·13	0·13	0·13	0·22	0·29
	39·3	0·243	0·36	0·29	0·25	0·24	0·26	0·31	0·35
	40·8	0·267	0·32	0·27	0·25	0·24	0·25	0·30	0·46

* Preliminary experiment at smaller concentration

For disulfoton and dimethoate, however, comparison with published values for molecules of similar size (e.g. in the International Critical Tables) suggests that D_L would be approximately 5×10^{-6} cm²/sec whereas D_G would be approximately 10^{-1} cm²/sec. The vapour concentration must therefore be very much less than the solution concentration before vapour movement can be neglected. Provided the vapour obeys Henry's law and with liquid pesticides, provided the liquid is not readily miscible with water, the ratio of the concentration in saturated aqueous solution to that in saturated air may be used to calculate the partition of pesticide between soil solution and soil air as suggested by Hartley.[9] Values of these partition coefficients would be approximately $2\cdot5\times10^{8}$ for dimethoate and $5\cdot5\times10^{3}$ for disulfoton. Vapour diffusion should therefore be insignificant for dimethoate. If it is assumed that the isotherm is linear, with slope b and the quantity in the vapour is negligible, then $C_L/C = 1/(bB+V_L)$, where B is the bulk density. Equation (4) may then be written:

$$D = D_L V_L f_L/(bB+V_L) \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (5)$$

Similar equations were derived by Call[1] for the diffusion of ethylene dibromide and by Olsen *et al.*[10] for diffusion of phosphate ions in soil, assuming diffusion was effectively through only one phase in the soil. The equation shows quantitatively how the different factors influence the value of the apparent diffusion coefficient; for example, the contribution of adsorption is determined by the value of b whereas f_L allows for the tortuosity of the pathway. Values of f_L found for dimethoate in a given soil should also apply for other pesticides that diffuse predominantly in solution. Values for B and V_L are known and b is obtained from the slope of the best fitting linear isotherm, so that if D_L is assumed to be 5×10^{-6}, f_L may be calculated at different moisture contents from the measured D values. Fig. 6 shows this for dimethoate. The values agree well with those for the equivalent 'transmission factor' found by Porter *et al.*[11] for diffusion of chloride in the Ca form of Pierre Clay soil over a smaller range of moisture content (Fig. 6). The shape of the curve resembles that found by Rowell *et al.*[12] for chloride diffusion in an Upper Greensand soil, although the values of f_L obtained for dimethoate are smaller as might be expected using the heavier Broadbalk soil. Because D_L was assumed for dimethoate, f_L values are only approximate, but comparison with these previous studies shows that the behaviour is consistent with diffusion predominantly in the liquid phase.

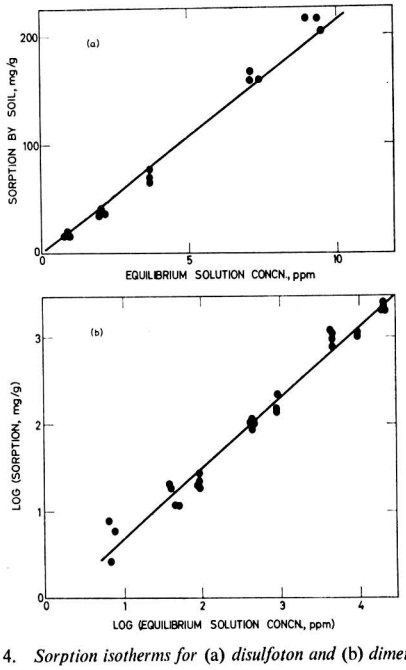

FIG. 4. *Sorption isotherms for* (a) *disulfoton and* (b) *dimethoate*

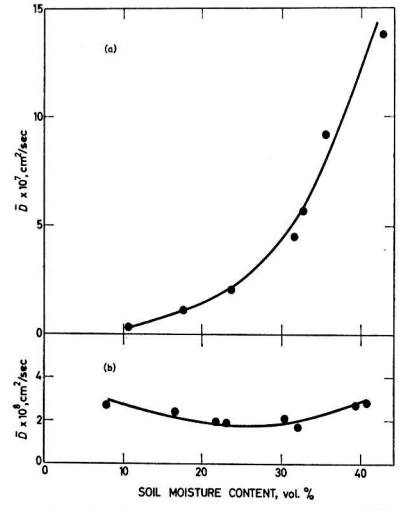

FIG. 5. *Effect of soil moisture content on average diffusion coefficients*
(a) Dimethoate; (b) disulfoton

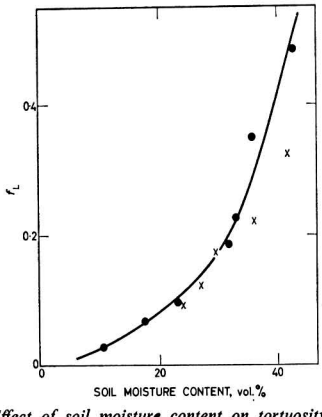

FIG. 6. *Effect of soil moisture content on tortuosity factors for dimethoate*
● Experimental points; × points from Porter *et al.*[11]

In contrast, moisture content has little effect on diffusion of the more volatile disulfoton and the contribution of vapour diffusion seems to increase with decreasing water content in approximately the same proportion as diffusion in solution decreases. The exact contribution of each phase is difficult to calculate. Rowell *et al.*[12] analysed the contributions of different pathways to the diffusion of ions in soil by equating the total flux with the sum of the fluxes in the different phases and estimating the liquid contribution by applying f_L values obtained for chloride which was assumed to move entirely in solution. Simple addition of fluxes in this way is questionable for pesticides if the fluxes in the two phases are comparable. It would be permissible if the soil consisted of independent air and water channels parallel to the direction of diffusion, analogous to a set of electrical conductors in parallel. Equally, in a hypothetical soil consisting of air and water laminae normal to the direction of diffusion, analogous to electrical conductors in series, the resultant flux would be zero if either component were non-conducting. Real soil is a complex mixture of these two extremes. The tortuosity factors f are empirical functions of this complexity and must be functions of both diffusivities, as well as of the geometry, unless one diffusivity is negligible. Values of f_L for dimethoate cannot therefore be used to estimate the solution phase contribution to the diffusion of disulfoton. However, the extent to which vapour movement for disulfoton modifies the movement of an involatile pesticide with the same adsorption characteristics may be seen by comparing the measured effect of moisture content on D with that on $D_L f_L V_L/(bB+V_L)$ calculated from the disulfoton sorption isotherm and using f_L values for dimethoate (Fig. 7). The calculated solution diffusion coefficient is a very small fraction of the observed value for total diffusion at small moisture contents, showing the pronounced effect of vapour movement. With increasing moisture, the estimated value increases rapidly and, in accordance with theory, agrees well with the measured value at the large moisture contents when vapour movement would be negligible. For the wettest soil, the calculated value in fact slightly exceeds the measured figure. In view of the assumptions made, this over-estimate is not large and may be attributed to errors in the assumed quantities. Alternatively, it may indicate that distribution coefficients (b values) obtained from equilibrium adsorption experiments with continuous shaking at large solution/soil ratios cannot be applied safely for diffusion calculations. Slow desorption could cause the effective distribution coefficients during diffusion to be larger than measured b values. The likely behaviour of other pesticides may be considered in the light of these results. The results obtained with dimethoate suggest that, provided f_L values are known, diffusion coefficients of relatively involatile materials may be calculated from independent measurements of sorption, moisture content etc., by using Equation (5). Although f_L values shown in Fig. 6 are only approximate, they are accurate enough to allow reasonable estimates of tortuosity effects for similar pesticides in this soil. Further, curves relating f_L to V_L for different soils seem to lie fairly close,[11] so that, in the absence of measurements for a given soil, f_L values reported here or elsewhere[11,12] could probably be used to indicate behaviour.

Estimating the additional vapour contribution to diffusion of more volatile pesticides is more difficult. However, the results for disulfoton suggest that for pesticides of similar volatility, values of D could be calculated by Equation (5) for wet soils where vapour movement was small and then assumed to change little over a wide range of moisture contents. Finally at the other extreme, diffusion in solution could be neglected for very volatile pesticides and diffusion coefficients estimated using the form of Equation (4) appropriate for vapour movement, such as that given by Call[1] for fumigants.

The measurements reported here emphasise that diffusion of both these insecticides is a relatively slow transport process whatever the soil moisture content. The root mean square displacement of the diffusing molecules (given by $(2Dt)^{\frac{1}{2}}$) would be at most about 2·5 cm in a month for dimethoate in the wettest soil studied, whereas the corresponding figure for disulfoton is about 0·3 cm. Diffusion also becomes less effective with time and cannot therefore transport pesticides of this type for long distances from where they are applied in soil. However, the differences in behaviour found could have important practical consequences for movement over short times and short distances, for example in the micro-regions around plant roots or granules in soil.

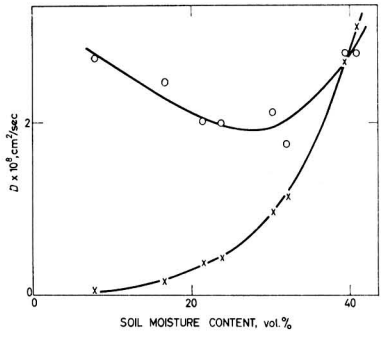

FIG. 7. *Comparison of measured diffusion coefficients for disulfoton with calculated solution diffusion coefficients*

○ Measured points; × calculated solution diffusion coefficients

Acknowledgments

The author wishes to thank Dr. G. S. Hartley for helpful discussions about diffusion in porous media. Technical disulfoton and dimethoate were kindly supplied by Messrs. Baywood Chemicals Ltd. and Fisons Pest Control Ltd.

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PESTICIDE RESIDUES IN FOODSTUFFS IN GREAT BRITAIN

XII.*—Organochlorine insecticide residues in hens' eggs from battery, deep-litter and free-range systems and from houses containing insecticide thermal vaporisers

By D. C. HOLMES, J. H. SIMMONS and J. O'G. TATTON

A study has been made of the organochlorine pesticide residues in hens' eggs produced in battery, deep-litter and free-range systems. Low residues (less than 0·05 ppm) were found except where the birds or their houses had been deliberately treated with pesticides to eradicate fleas or lice.

A separate study was also made of eggs produced in houses which had insecticide thermal vaporisers installed in them. The results were variable, depending on a number of factors, but relatively high levels of BHC and DDT were found regularly in the eggs from some of these houses.

Introduction

In recommending that a study be made of the organochlorine pesticide residues in the eggs of domestic hens, the Panel on Residues of Pesticides in Foodstuffs[1] was concerned with two distinct aspects.

Firstly, it was considered desirable to ascertain the general range of the pesticide content of eggs. The East Craigs Laboratory (Department of Agriculture and Fisheries for Scotland) had made an earlier study[2] of eggs and poultry from an area restricted to southern Scotland and northern England. The samples were taken from battery, deep-litter and free-range systems and, in general, all the residue levels found were low. However, none of the establishments sampled in this previous study was using pesticides in the course of egg production, whereas in other areas, such as southern England, it was known that pesticides were frequently used to control ectoparasites. For this reason, the Panel thought it desirable to extend the survey to the rest of the U.K. It is worth noting in this context that about 67% of the eggs currently produced in the U.K. come from battery houses. Deep-litter establishments account for another 25% and only 8% come from free-range birds.

Secondly, it was thought that some investigation should be made into the effect of the use of insecticide thermal vaporisers in chicken houses on the residue levels occurring in eggs produced under those conditions. The insecticide thermal vaporisers normally used in chicken houses consist of a metal cup into which tablets of pesticide are placed and which is heated by a small thermostatically controlled electric element. When in operation, the heater maintains the insecticide in a molten state so that there is continuous emission of the volatilised insecticide into the atmosphere. The quantities evolved are dependent on the operating temperature but are not otherwise controlled.

Some of the effects of these vaporiser units have already been examined. As early as 1956, Siakotos[3] in the U.S.A. showed that some packaged foods contained significant amounts of gamma-BHC after exposure to vapours generated by such units, containing this pesticide, in a basement food store. Five years ago, Dyte[4] in this country drew attention to the contamination of uncovered foodstuffs which could occur if they were exposed to insecticide vapours. His experiments were conducted in closed rooms with units emitting a vapour of either gamma-BHC or DDT. The County Analyst for Somerset[5] has investigated some of the effects of these vaporiser units in food shops; deposits on shelves and other surfaces were analysed and residues in foodstuffs on the shelves were determined.

Some investigations have been conducted into the residues in hens' eggs after exposure of the birds to insecticidal vapours from tablets attached to the wire floors of battery cages.[6] These tablets were designed to control ectoparasites by slow vaporisation at ambient temperatures. No work so far appears to have been carried out to determine the residues occurring in hens' eggs as a consequence of the use of thermal vaporisers in the houses.

Experimental

Samples

In the general study, advantage was taken of an existing egg sampling scheme designed to compare the nutritional values of intensively and extensively produced eggs. This scheme was organised by the Ministry of Agriculture, Fisheries and Food with the assistance of Dr. M. J. Head (University of Surrey), under the auspices of the Interdepartmental Committee on Food Composition. The results of this comparison will be published separately. The sources of these eggs were 7 agricultural institutes, or farms attached to agricultural colleges or universities, and were widely distributed in the U.K. including one in Scotland and one in Northern Ireland. At these establishments, eggs were produced in battery, deep-litter or free-range systems typical of those in commercial production. Monthly samples were submitted from at least two of these systems from each establishment for a period of a year. So far as was known, insecticide thermal vaporisers were not in use at any of these establishments.

Six farms which had thermal vaporisers installed in their chicken houses were also selected; all were situated in southern England and were engaged in commercial egg production. Three of these farms also had other houses which did not have such devices installed and these provided useful control samples. Samples of eggs were taken monthly at these farms over periods ranging from 6 to 9 months. At the end of the sampling period, which was usually determined by the farmer's

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decision to slaughter and re-stock, a visit was paid to each farm, and various other samples were taken. These included, where appropriate, samples of feed exposed in the houses, feed from store, dust on floors, litter, fluff from ceiling and beams, etc. The contents of the vaporiser units were also sampled for identification of the pesticides used.

Methods of analysis

In breaking the eggs for analysis, care was taken to avoid contaminating the contents with any pesticide that might have been on the outside of the shell. Usually the contents of 3 or 4 eggs were whisked together to provide a sample for analysis. 5 g of this sample were mixed in a beaker with an equal weight of granular anhydrous sodium sulphate and a little sharp sand to provide a friable mixture. This mixture was warmed and stirred on a steam bath for about 5 minutes with 4 successive portions of 25 ml propan-2-ol. The alcoholic layer from each extraction was decanted and the combined extracts, after cooling, were bulked to 100 ml with propan-2-ol. 50 ml of this solution were mixed in a 500 ml separator with 50 ml hexane; 300 ml 4% sodium sulphate solution were added and the mixture was shaken for 2 minutes. After the two phases had been allowed to separate for 15 minutes, the lower aqueous phase was discarded and the hexane layer was dried by being passed through a short column of anhydrous sodium sulphate, the column being washed through with another 10 ml hexane. The hexane eluate was then cleaned-up by a dimethylformamide–hexane partition method[7] followed by passage through a column of prepared alumina (alumina heated at 500° for 4 hours, cooled, and partly de-activated by the addition of 7% of water) using hexane as the eluting solvent. The final eluate was concentrated and examined by gas chromatography using silicone and Apiezon columns with electron-capture detection.[8]

This method was shown to give recoveries, from eggs, of 90% or more for all the common organochlorine pesticides and their metabolites or immediate breakdown products. Limits of detection were of the order of 0·005 ppm or better for these compounds.

Some of the samples of dust, fluff and litter required only a simple extraction with hexane to provide a solution which, after suitable dilution with hexane, was sufficiently clean to allow direct analysis by the same gas chromatographic method. Other samples, including feed, required a clean-up as described above before also being subjected to gas chromatographic analysis.

Results

The compounds most frequently detected in the egg samples were gamma-BHC, pp′-DDT, and pp′-DDE, which is the first toxic metabolite or breakdown product of DDT. Traces of beta-BHC, op′-DDT, and pp′-TDE were found in a few samples but usually these were less than 0·01 ppm. Dieldrin was frequently detected at about 0·01 ppm; only in eggs from Farm D was that figure significantly and regularly exceeded and this will be referred to later. No other organochlorine pesticide residues were detected in the eggs.

Table I summarises the results for the eggs obtained from the 7 agricultural institutes and farms attached to colleges and universities. Separate figures are given for battery, deep-litter and free-range eggs.

The results for eggs from the two intensive systems, battery and deep litter, are not markedly different, either as regards mean values or range of values. 85% of the eggs from the battery houses and 65% of those from the deep-litter houses contained no pesticide residue in excess of 0·05 ppm. The majority of the remaining results were sufficiently high to justify belief that the birds had in some way come into direct contact with pesticides. These high results were confined to 3 farms, and enquiries revealed that in all cases, DDT or BHC had been used in the houses during the sampling period to control infestations of fleas or lice. The farms confirmed that insecticide thermal vaporisers were not used, but that birds and houses had been dusted or similarly treated.

TABLE I

Pesticide residues in eggs from agricultural institute farms, etc., without thermal vaporisers

Mean values and ranges in ppm

System	Samples		α-BHC	pp′-DDE	pp′-DDT
Battery	86	mean	0·02	0·02	0·03
		range	(0–0·30)	(0–0·20)	(0–0·37)
Deep-litter	54	mean	0·04	0·02	0·04
		range	(0–0·33)	(0–0·13)	(0–0·31)
Free-range	33	mean	0·04	0·36	0·54
		range	(0–0·40)	(0·01–2·8)	(0–3·8)

The results for the free-range eggs show much higher mean levels for DDT compounds. Nevertheless, nearly 60% of these eggs contained residues which did not exceed 0·05 ppm. 3 samples contained between 0·12 and 0·40 ppm gamma-BHC but 8 samples contained between 0·15 and 3·8 ppm pp′-DDT, accompanied by between 0·12 and 2·8 ppm pp′-DDE. Again 3 farms were found to be responsible for all the eggs having these comparatively high residue levels in them and again it was discovered that these pesticides had been used during the sampling period to counteract lice by dusting the birds or their nests.

Table II gives the results for eggs from the 6 farms which had insecticide thermal vaporiser units installed in the chicken houses. Where the farms also had other houses without vaporiser units, the results of the analysis of eggs from these other houses are also given for comparison. In view of the wide differences between the results from these farms, they are best dealt with individually.

Farm A

This farm was a large deep-litter establishment producing 'broiler' chickens with eggs as a by-product. The houses were of asbestos and concrete construction of about 1300 cubic metres capacity. Each house was divided into six sections by wire mesh screens, each section being ventilated by a cross-flow forced draught.

Only three of the houses had thermal vaporisers installed. In these houses, 3 large vaporisers, each serving 2 wired sections, were mounted about 25 cm from the floor. The material in the vaporisers was a mixture of about 83% DDT and 17% gamma-BHC. The results show that mean residue levels in the eggs produced in these houses rose steadily from 0·05 ppm gamma-BHC and 0·13 ppm total DDT compounds at the beginning of the 5-month sampling period, to 0·08 ppm gamma-BHC and 0·52 ppm total DDT compounds at the end of the period, with some samples showing about 1 ppm total DDT compounds. By contrast the eggs produced in houses which did not contain thermal vaporisers showed residues of the order of 0·01 ppm

Feed for the hens in these houses was in pellet form and reached the birds by an automatic transmission system.

Pesticide residues in the feed in both kinds of house were of a low order – 0·01 to 0·02 ppm beta-BHC, gamma-BHC and pp′-DDT, the feed in the treated houses containing only marginally higher amounts than feed in the other type of house. Litter dust on the floor of the treated houses contained about 50 ppm gamma-BHC and about 190 ppm DDT compounds of which 130 ppm was pp′-DDT, 50 ppm was op′-DDT and 9 ppm was pp′-DDE and pp′-TDE. Residues in the dust on the floor of untreated houses were below the 0·01 ppm level. An area of wall in one of the treated houses was cleaned with a hexane-soaked swab; the residues in the dust removed were equivalent to 1700 μg pp′-DDT, 170 μg op′-DDT and 50 μg of gamma-BHC per square metre of wall.

Farm B

This farm had battery hens only, in small well-ventilated houses of about 425 cubic metres capacity. Each house had 2 thermal vaporiser units installed on opposite walls about 2 metres above the floor. The material in the vaporiser units was found to be a mixture of 95% DDT and 5% gamma-BHC. Over the 6-month period of sampling at this farm, the residue levels in the eggs remained reasonably constant from month to month. The mean level of DDT compounds (0·08 ppm) was significantly higher than the general levels in eggs from untreated houses but is low compared with other results obtained in similar establishments. The main stock of feed in a storage bin contained 0·01 ppm gamma-BHC and 0·06 ppm total DDT compounds. Feed in an open feeding trough in the house contained 0·11 ppm and 0·14 ppm respectively of these pesticides. Thick dust on a wall was heavily contaminated with these pesticides, equivalent to about 24 g gamma-BHC, 10 g pp′-DDT and 4 g op′-DDT per square metre of wall.

Farm C

There were two types of house at this farm. The deep-litter houses containing the thermal vaporisers were old stone-and-wood converted farm buildings, somewhat ramshackle, with large internal areas separated by wire or matchboarding from floor to ceiling. The section from which the egg samples were taken was about 30 cubic metres in volume. It contained one thermal vaporiser mounted about 1 metre above the litter surface. The material in the vaporiser was 55% gamma-BHC and 45% DDT. The construction of the building was such that even with all the 'doors' and 'windows' closed, there would still have been a fair degree of ventilation. The other house at this farm was a modern well-ventilated battery house with the hens in small wire cages in racks. No thermal vaporisers were installed in this house.

The results from the eggs at this farm showed the usual low residues in the untreated house but relatively high residues of gamma-BHC and DDT compounds in the eggs from the treated house. At the beginning of the sampling period the mean residues in the eggs from the latter house were 0·16 ppm gamma-BHC and 0·32 ppm DDT compounds. Over the next 8 months these increased steadily to 0·60 ppm gamma-BHC and 0·75 ppm DDT compounds at the end of the period. Samples of feed, in coarse powder form in open troughs in the treated house contained between 0·14 and 0·20 ppm gamma-BHC and 0·02 to 0·05 ppm pp′-DDT, compared with 0·03 ppm and 0·01 ppm respectively in similar feed in the untreated house. Litter from the deep-litter floor contained 10 ppm gamma-BHC and 5 ppm DDT compounds. Loose dust on the wall of the litter house contained residues equivalent to about 500 μg gamma-BHC and 250 μg DDT compounds, including 217 μg pp′-DDT, per square metre of wall. By comparison only negligible residues of pesticides were found in the dust on the floor of the battery house.

Farm D

All the houses at this farm were deep-litter houses. The house from which samples were taken was of about 2500 cubic metres capacity, equipped with 7 thermal vaporisers mounted 2 metres above the floor. These vaporisers contained a mixture of 63% gamma-BHC and 37% DDT. Eggs at this farm were sampled monthly over a period of 9 months during which time the mean gamma-BHC content of the eggs was fairly constant at about 1·6 ppm but the total DDT compounds rose from 0·11 ppm to 0·28 ppm. Feed from suspended open bins in the house contained 0·29 to 0·32 ppm gamma-BHC and 0·03 to 0·06 ppm pp′-DDT compared with 0·04 ppm and 0·02 ppm respectively in the main stock outside the house. One feature of the house was the long white trails and pendants of fluff hanging from beams, wooden walls and wire mesh. These often became detached by the draught when a door was opened and settled on the birds or litter. A collected sample of this fluff contained 5·9% gamma-BHC, 0·16% pp′-DDT and 0·02% op′-DDT. An unusual and disturbing feature of the analysis of the eggs from this farm was the constant detection of dieldrin in amounts significantly

TABLE II

Pesticide residues in eggs from farms using insecticide thermal vaporisers

Mean values and ranges in ppm

Farm	Eggs from houses containing thermal vaporisers				Eggs from houses without thermal vaporisers			
	Samples	gamma-BHC	pp′-DDE	pp′-DDT	Samples	gamma-BHC	pp′-DDE	pp′-DDT
A	15	0·05 (0·03–0·08)	0·10 (0·03–0·33)	0·23 (0·07–0·75)	13	<0·01 (0–0·01)	0·01 (all 0·01)	<0·01 (0–0·01)
B	28	0·01 (0–0·03)	0·03 (0·01–0·07)	0·05 (0·03–0·13)	—	— —	— —	— —
C	32	0·28 (0·07–0·65)	0·20 (0·06–0·50)	0·28 (0·08–0·60)	32	<0·01 (0–0·02)	0·01 (0·01–0·02)	0·02 (0–0·03)
D	28	1·6 (0·90–2·1)	0·07 (0·02–0·15)	0·13 (0·03–0·25)	—	— —	— —	— —
E	30	0·43 (0·05–1·3)	0·05 (0·02–0·16)	0·15 (0·04–0·45)	29	0·01 (0–0·03)	0·01 (0·01–0·05)	0·01 (0·01–0·03)
F	14	0·02 (0–0·06)	0·02 (0·01–0·03)	0·04 (0·01–0·09)	—	— —	— —	— —

higher than the usual level in eggs, of 0·01 ppm or less. The mean dieldrin content of the eggs from this farm over a period of 5 months was 0·13 ppm with a range of from 0·05 to 0·30 ppm. A can of disinfectant in use at the farm was found to contain 0·25% wt./vol. of dieldrin. It was the practice to spray the litter and lower part of the walls with the diluted disinfectant at regular intervals. Examination of the litter showed it to contain 0·30 ppm dieldrin. This type of disinfectant has now been withdrawn from sale.

Farm E

All the hens at this farm were battery birds in wire cages in wooden houses of about 700 cubic metres capacity. Some of these houses contained thermal vaporisers and, where they were installed, the farmer had paid strict attention to the advice of the suppliers and mounted 3 on the floor of each house. The mixture in these units, at the time of sampling, was 74% DDT and 26% gamma-BHC.

All the eggs from the untreated houses contained the usual low residues of 0·01 ppm or less of gamma-BHC and DDT compounds throughout the 9-month sampling period. By contrast, the eggs from the treated houses contained significantly higher residues of gamma-BHC but they fluctuated fairly widely over the sampling period. Mean values for individual months varied from 0·07 to 1·0 ppm gamma-BHC, and from 0·09 to 0·49 ppm total DDT compounds. Enquiry revealed that the farmer varied the speed of his ventilation fans according to the weather. This is a common practice in battery houses but, in this case, it had a marked effect in that during a long cold spell the residue levels in the eggs were high while in warmer months lower residue levels occurred in the eggs. Samples of feed from the treated houses contained from 0·33 to 0·65 ppm gamma-BHC and from 0·05 to 0·09 ppm DDT compounds compared with up to 0·05 ppm and 0·01 ppm respectively in untreated houses. Dust and fluff from the treated houses contained up to 0·56% gamma-BHC and 0·07% DDT, but no pesticides were detected in dust and fluff in the other houses.

Farm F

The unexpected sudden sale and closure of this farm meant that sampling and information about these premises were limited. The farm contained a single large battery house served by 2 thermal vaporisers mounted about 2·5 metres above the ground. The material in the vaporiser units was a mixture of DDT and gamma-BHC but the proportions are not known. Some of the results for the eggs show definite evidence of contact with both these pesticides but the results as a whole are low. It was established that the vaporiser units had been installed fairly high up and close to the extractor ventilation fans. It is possible, therefore, that most of the insecticide vapour was quickly removed by the fans.

Conclusions

Residues of organochlorine pesticides in eggs are usually of a very low order, provided that the birds have not come into direct contact with the pesticides. This applies whether the birds are kept in battery, deep-litter or free-range establishments. Normally beta-BHC, gamma-BHC, dieldrin, pp′-DDE and DDT may be detected in such eggs up to about the 0·01 ppm level, the same level at which the compounds appear to be present in the commercial feeds examined. These results, in fact, are similar to those reported in the earlier study.[2]

Very much higher levels of gamma-BHC and DDT compounds can occur in the eggs of birds which are either directly treated with these pesticides in attempts to eradicate fleas or lice, or which are kept in houses where they are subjected to these pesticides from thermal vaporiser units. In the first case the birds probably receive an initial comparatively large dose which may be reinforced if nests and houses are also treated, but the effects of which will wear off, so that residues in the eggs should decrease with time. In the second case there is continuous contact with pesticide in the atmosphere, on coated surfaces and by way of pesticide-contaminated feed. Thus, as has been seen at some farms, the residue levels increase in the eggs the longer the birds are retained under these conditions.

However, the results for the eggs from chickens kept in houses with insecticide thermal vaporisers show considerable variation from farm to farm. There is no doubt that the number of such vaporiser units per house, their situation in the house and the ventilation arrangements can play a big part in determining the residues that will occur in the eggs. It is significant that all the farmers using these units expressed satisfaction with their effects in keeping down or eradicating insect infestations, even at those farms where residue levels were very low.

Technical DDT usually consists of about 80% pp′-DDT and 20% op′-DDT. The DDT in the vaporiser units was always in the form of this mixture of isomers, and both isomers were detected in the feed and other materials sampled. Nevertheless, op′-DDT was rarely detected in any of the eggs and never at a level exceeding 0·01 ppm. It is clear that hens can metabolise op′-DDT much more efficiently than pp′-DDT. Some evidence of the conversion of op′-DDT to pp′-DDT in cocks has been reported.[9]

Acknowledgments

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DEGRADATION OF COMMERCIAL DDT IN SILAGE

By R. F. HENZELL and R. J. LANCASTER

The fate of DDT present in ensiled pasture herbage has been studied in laboratory silos of 2 kg capacity. The DDT was extensively decomposed in the process, but the accumulation of equally objectionable degradation products, identified as pp′-DDD and op′-DDD gave a net reduction of about 50%. These changes appeared to be unrelated to the type of silage fermentation that occurred. The results suggest ensilage to be a useful management procedure in dealing with DDT-contaminated pasture.

Introduction

In many permanent pasture areas of New Zealand, infestation of the soil by insect pests is sufficient to cause partial, or occasionally complete, destruction of the grass. Attempts to control infestation have usually been by surface application of DDT. Animals grazing in treated areas may, however, ingest DDT in amounts which will cause contamination of the meat or dairy produce, to the extent that these may not meet tolerance levels set for human consumption. Contamination can be reduced to acceptable levels by the use of special techniques for applying the insecticide, and by grazing management. The most positive measure, however, would be to degrade DDT and its harmful derivatives on pasture to non-toxic products before it is fed to the animals.

Numerous investigations have been carried out on the fate of DDT as it degenerates in biological systems. It has been shown[1–3] that DDT can be degraded to DDD by bacteriological action. Thornburg[4] has briefly reported the conversion of DDT to DDD in maize silage, but made no reference to the type of fermentation involved.

It appears therefore that the silage process might be an effective method for reducing residues on pasture contaminated with DDT. 'Silage process' covers a wide range of biological conditions, however, and it is possible that DDT might be degraded differently in the environments of different silage fermentations. This paper presents the results of a series of experiments undertaken to examine the degradation of DDT present in ensiled pasture herbage. The specific objectives of the experiments were: to measure the extent of degradation of DDT, to characterise the derivatives of DDT appearing in silages and to measure the extent of the accumulation of these derivatives, and to assess the effect of the type of silage fermentation on the degradation of DDT.

Experimental

Field work

Two herbage types were selected, one mainly ryegrass and the other a cocksfoot–clover–ryegrass mixture, on different sites, 60 × 20 ft in area. The pasture was mown to a height of 3 in and both sites were fenced. Commercial DDT was applied to half the area at each site, in 50% water suspension with a manual boom spray delivering 2 lb DDT/acre. The commercial material used contained 75·8% pp′-DDT, 23·8% op′-DDT, 0·4% pp′-DDE and 0·015% pp′-dichlorodiphenyl monochloroethylene (pp′-DME or pp′-DDMU). The unsprayed areas produced control material for each trial.

Preparation of silages

The silage fermentations were conducted in laboratory 'vacuum' silos consisting of a 12 × 18 in polythene bag fitted with a PVC valve for evacuation. The open end of the bag was sealed with 'strip seal'.[5]

15 kg of herbage were harvested from treated and control areas at both sites after 14 and 35 days and at the 'cocksfoot' site after 70 days. Each 15 kg batch was chopped as short as possible (1–2 in), thoroughly mixed on polythene, and distributed into 6 polythene bags until each bag contained 2 kg. The contents of each bag were re-mixed. Four of the bags were placed in laboratory vacuum silos evacuated to 15 in Hg and kept in duplicate in incubators at 24° and 38°. In the final trial the material destined for the 38° incubator was left exposed to the air for 24 h to increase the difference between fermentation types expected between 24° and 38° silages. Silage fermentations were maintained for 90 days. Contents of the other two bags were used for chemical analysis.

Sampling

Sampling was carried out by removing the inner bag, and taking cores with a ½ in (i.d.) steel tube. The silo was then re-assembled and re-evacuated. Provision was made for the collection of juice during evacuation.

During the fermentation, two 10 g core samples were taken for DDT analysis from each silo on the days indicated (Figs 1–5). At the same time, core samples were taken for silage acid and pH measurements.

In view of the anticipated large sampling error, the variation of DDT in 10 g core samples was determined by measuring DDT in ten 10 g core samples taken from each bag reserved for chemical analysis. The results of this study are given in Table I.

The data indicate that large sampling errors might be expected from pairs of core samples taken from the various silos during fermentation. These errors precluded the quantitative study of the problem, but the data obtained from sequential samples were satisfactory for the qualitative appraisal of the situation. Concentrations of DDT and derivatives in the final (90 day) silages were determined on five 100 g samples.

TABLE I

Variability of DDT concentrations in core samples

Bag means: 10 samples/bag

DDT, ppm in dry matter

Trial No.	Bag 1	Bag 2	Difference	Standard deviation	Coefficient of variation, %
1	266	266	n.s.	34	13
2	468	536	n.s.	159	32
3	63	52	*	11·2	19
4	51	46	n.s.	13·9	29
5	2·8	3·2	n.s.	0·60	20

* Significance at 5% level

Analytical

Gas–liquid chromatography (g.l.c.)

Gas chromatograms of DDT derivatives were obtained with an F & M model 400 gas chromatograph, equipped with an electron capture detector. A 1/6 in × 6 ft glass U-tube column consisting of 1·5% SE 30 on Embacel 60–80 mesh support DDS-treated, was used. The nitrogen flow rate was 90 ml/min. The column, flash heater and detector temperatures were 195°, 250° and 205° respectively.

Since the column used could not separate all derivatives, it was necessary to make a preliminary separation by thin-layer chromatography.

Infra-red spectrophotometry

Infra-red spectra were obtained using a Beckman IR 8 spectrophotometer equipped with a beam condenser. Micropellets were prepared, using ~ 40 μg sample to 1–2 mg KBr. The sample–KBr mixture was ground in an agate mortar under evaporating methanol and the pellets were obtained using an R.I.I.C. H 30/1, 30 ton press.

Chemical analysis of insecticide

Each 10 g core sample was extracted with hexane–isopropanol (3 : 1). Using this solvent[6] the recovery of DDT added to control pasture was 90–95%. A 5 ml aliquot of the hexane extract was pipetted on to 2 g Florisil topped with 1 g sodium sulphate in a chromatographic column. All the DDT derivatives were eluted from the column using 20 ml 15% by vol. dry ether in hexane. The solution was made up to 25 ml and 10 μl were injected on to the g.l.c. column. If the g.l.c. analysis showed a number of derivatives to be present, an aliquot of the solution containing ~ 3 μg of the derivatives was evaporated to about 10 μl in a micro Kuderna-Danish evaporator. The derivatives, separated by thin-layer chromatography using n-hexane on silica gel chromatoplates,[7] were extracted from the silica gel with hexane using the method of Harrison[8] before injection on to the g.l.c. column.

Silage analysis

Dry matter in silage was measured by toluene distillation.[9] Total N was determined as ammonia in Kjeldahl digests by means of a Technicon Autoanalyser. Free ammonia was determined in aqueous extracts of fresh silage samples by Conway micro-diffusion.[10] Silage acids were estimated in extracts prepared as detailed by Wiseman & Irvin.[11] The acids were separated in silicic acid columns and estimated as described by Lessard & McDonald.[12]

Identification of derivatives

Approximately 10 kg of herbage from the treated area were placed in a large laboratory vacuum silo and incubated at 38° for ~ 40 days. The material was extracted with hexane–isopropanal and after the polar solvent had been partitioned into water, 7 l of hexane solution remained, and this was reduced to about 150 ml on a rotary vacuum evaporator. A third of this concentrate was cleaned up on a Florisil adsorption column (60–100 mesh, 35 g, 18 × 2·4 cm) using hexane (200 ml) followed by 15% by vol. ether–hexane (250 ml) as eluting solvents. The solution was then concentrated to 5 ml using a Kuderna-Danish evaporator, and the process was repeated three times.

The concentrate was evaporated under a stream of air almost to dryness and extracted five times with acetonitrile saturated with hexane. The extracts were combined and partitioned three times with hexane saturated with acetonitrile. The acetonitrile phase, containing the DDT derivatives was evaporated to dryness, and the residue was taken up in ethanol and recrystallised with activated charcoal.

This crude material was divided equally. One portion was recrystallised three times from aqueous ethanol. Gas chromatographic analysis indicated the product to be approximately 90% pp′-DDD and 5% op′-DDD. An infra-red spectrum of the sample agreed precisely with the spectrum of pp′-DDD described by Morris & Haenni.[13] Characteristic absorption bands at 806, 763 and 752 cm^{-1} were observed.

The second portion (1·5 mg) was recrystallised three times from hexane. The final product (~ 60 μg) in hexane was analysed by gas chromatography and contained about 85% op′-DDD, 15% pp′-DDD. An infra-red spectrum was obtained and agreed precisely with the spectrum of pure op′-DDD. Characteristic absorption bands at 609, 1034–35 (doublet) and 1472–73 cm^{-1} (doublet) were observed.

Results and Discussion

Description of silages

As similar fermentation data were obtained from DDT treated and control silages only those from treated material will be considered. The control silages provided blank material for detailed studies of DDT and its products.

TABLE II

Silage characteristics (90 days)

Trial No.	Temp., °C	Dry matter, %	Total N*	NH_3-N, % total N	pH	Lactate*		Butyrate*	Acetate*	Stability
						1†	2†			
1	24	19·5	4·83	11·0	5·1	4·0	0·0	0·0	10·0	Intermediate
	38	19·1	5·00	25·5	6·5	3·2	0·0	6·3	6·5	Unstable
2	24	16·1	3·42	16·0	5·3	4·2	0·0	1·1	5·4	Unstable
	38	13·4	4·47	15·3	5·7	5·6	0·0	4·0	5·3	Unstable
3	24	23·7	3·37	6·7	3·9	3·9	8·4	0·0	3·5	Stable
	38	18·4	4·68	21·0	5·9	6·5	0·0	2·0	4·2	Unstable
4	24	22·6	2·84	8·2	3·8	4·8	7·6	0·0	1·8	Stable
	38	21·3	3·00	17·8	5·1	4·0	0·0	0·9	5·6	Unstable
5	24	18·4	3·42	10·2	4·6	4·3	0·0	0·0	7·6	Intermediate
	38	18·5	3·66	24·3	6·0	1·0	0·0	3·2	6·4	Unstable

* As % of dry matter

† Lactate 1 refers to first sequential sample; lactate 2 refers to final (90 day) sample

In all fermentations, lactic acid production was satisfactorily initiated (Table II) but considerable differences in subsequent behaviour developed. In every 38° silage, high values were recorded for pH and ammonia. Butyric acid was found in all 38° samples but no lactic acid was detected. These can be characterised as unstable clostridial-type fermentations.

The 24° silages exhibited variable fermentations. Trial 2 was similar to its counterpart at 38°. Trials 3 and 4 quickly achieved low pH and exhibited the characteristics of highly stable silages. Trials 1 and 5 were intermediate in character. Both were high in acetic acid concentrations and contained negligible amounts of the other acids. The pH values were higher than are generally accepted for stable silages, but the low ammonia values indicated little putrefaction. The results may be summarised by stating that the DDT studies were made under four different conditions of silage fermentation: stable at 24°, intermediate stability at 24°, unstable at 24° and unstable at 38°.

Changes in concentrations of DDT and derivatives

Changes in concentration of pp′-DDT and of its main derivative pp′-DDD are shown in Figs 1–5. It should be noted that concentrations of op′-DDT and op′-DDD generally paralleled those of their isomers pp′-DDT and pp′-DDD.

With the exception of trial 1 (Fig. 1), the patterns of change were similar at the two temperatures in each trial, though there were differences in those patterns between trials. In trials 1 (at 38°), 2, 4 and 5 there were rapid and substantial decreases in pp′-DDT concentrations; in trial 1 (at 24°), and 3, pp′-DDT concentrations remained essentially unchanged during the sequential sampling period (23 days) and later decreased.

These changes in DDT were accompanied by the development of derivatives (Figs 1–5). Because of the large sampling error of the core samples, changes in DDT concentrations cannot be reliably interpreted. The appearance of DDT derivatives is therefore taken as a surer indication of DDT breakdown than decreases in pp′-DDT concentrations.

There appeared to be no relation between the DDT or DDD changes recorded in Figs 1–5 and the fermentation characteristics of the silages. Although the fermentations at the two temperatures in trials 3, 4 and 5 were markedly different, the similar patterns of change of DDT at these temperatures in each case suggest the operation of factors common to both temperatures.

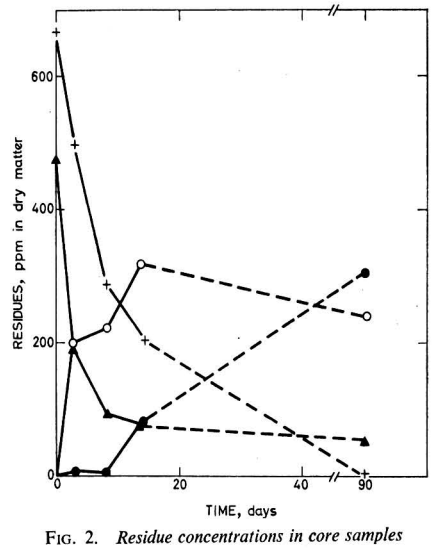

FIG. 2. *Residue concentrations in core samples*
+ pp′ DDT at 24°C; ● pp′ DDD at 24°C; ▲ pp′ DDT at 38°C; ○ pp′ DDD at 38°C
24°C Unstable; 38°C unstable

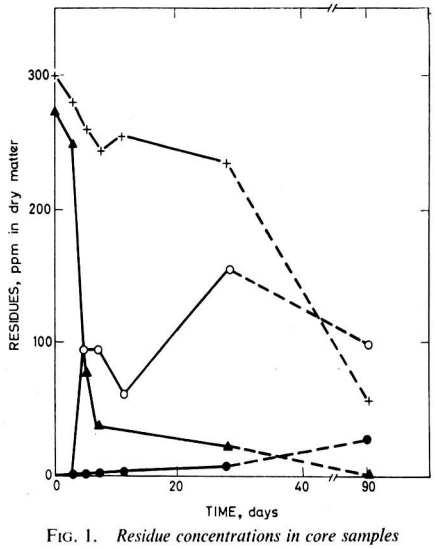

FIG. 1. *Residue concentrations in core samples*
+ pp′ DDT at 24°C; ● pp′ DDD at 24°C; ▲ pp′ DDT at 38°C; ○ pp′ DDD at 38°C
24°C Intermediate; 38°C unstable

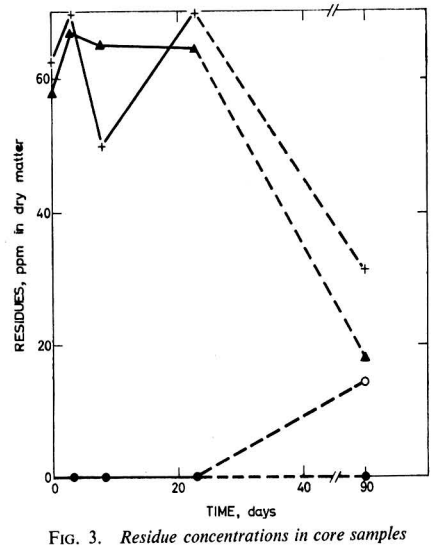

FIG. 3. *Residue concentrations in core samples*
+ pp′ DDT at 24°C; ● pp′ DDD at 24°C; ▲ pp′ DDT at 38°C; ○ pp′ DDD at 38°C
24°C Stable; 38°C unstable

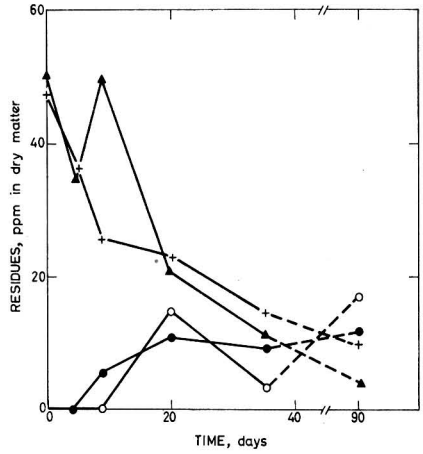

FIG. 4. *Residue concentrations in core samples*
+ pp′ DDT at 24°C; ● pp′ DDD at 24°C; ▲ pp′ DDT at 38°C; ○ pp′ DDD at 38°C
24°C Stable; 38°C unstable

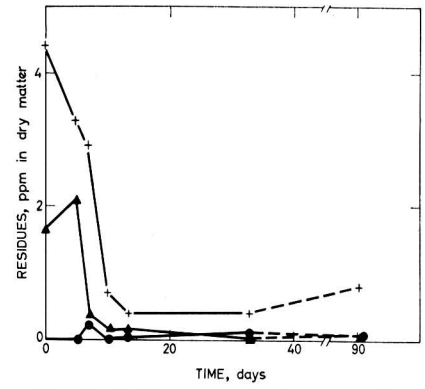

FIG. 5. *Residue concentrations in core samples*
+ pp′ DDT at 24°C; ● pp′ DDD at 24°C; ▲ pp′ DDT at 38°C; ○ pp′ DDD at 38°C
24°C Intermediate; 38°C unstable

Table III presents the levels of DDT and derivatives in ensiled herbages and in the final silages (90 days). It will be seen that about 75% of ensiled DDT disappeared from the 24° silages whilst about 90% disappeared at 38°.

These changes were partly compensated by the appearance of DDD and the net change is seen to be remarkably similar at the two temperatures in each trial. The net reduction in insecticides was of the same order, about half the original level, in the four different types of fermentation developed. Only trace quantities of residues were detected in some juice samples.

Conclusions

The object of this work was to discover whether pasture contaminated with DDT could be improved by ensilage, and whether conditions of ensilage were a factor in this connexion. It has been shown that residues can be reduced by about half for all four different ensilage conditions studied. This is a substantial gain and indicates the usefulness of ensilage in the amelioration of contaminated pasture.

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TABLE III
Mean values DDT and derivatives

Trial No.	Temp., °C	Herbage, ppm in dry matter		Silage, ppm in dry matter					% Residues removed in silage process
		pp′-DDT	op′-DDT	pp′-DDT	op′-DDT	pp′-DDE	pp′-DDD	op′-DDD	
1	24	266	39	64	8	3	32	6	62
	38	266	39	1	0	0	98	17	62
2	24	502	51	29	3	2	307	57	28
	38	502	51	2	1	4	241	43	27
3	24	58	9	31	5	0	1	0	45
	38	58	9	18	3	0	15	4	40
4	24	48	6	10	1	0	12	2	54
	38	48	6	4	0	0	17	4	54
5	24	3·0	0·7	0·8	0·2	0	0·1	0	70
	38	3·0	0·7	0·1	0·1	0	0	0	90

FUNGICIDES IN FORESTRY IN GREAT BRITAIN*

By D. H. PHILLIPS

In Great Britain, fungicides are used in the forest only for the control of root and stem rot, caused by *Fomes annosus*. In forest nurseries they are employed on a small scale to control damping-off, grey mould (*Botrytis cinerea*), needle-cast of pine caused by *Lophodermium pinastri*, needle-cast of larch caused by *Meria laricis*, needle blight of Western red cedar (*Thuja plicata*) caused by *Didymascella thujina*, and oak mildew (*Microsphaera alphitoides*).

Forest disease control

Fomes annosus[1] is the most damaging of all the fungi that attack forest trees in Britain. Its host range is wide, though it is commercially important only on conifers. It may kill all commonly planted conifers when they are very young; older trees, particularly pines, may also be killed on some sites. In other species (including Sitka spruce, the most important commercial tree) it rots roots and causes extensive stem rot. The root rot then often renders the trees liable to be blown down by wind.

The fungus enters healthy plantations mainly through fresh stumps left after thinning of the crop. Its airborne spores colonise the stump, and it then grows down into the roots, and by root contacts attacks the roots of adjacent healthy trees. To prevent this stump colonisation, freshly exposed stump surfaces are now painted with chemicals, though in the case of pine, a spore suspension of the competing fungus *Peniophora gigantea* is often used instead.[2]

Of the chemicals the first to be used was creosote.[3] This gave fairly good results, but it has a number of disadvantages: it excludes all competing fungi from the stump surface, so that if *F. annosus* is already present in the roots below, it may spread unchecked into the body of the stump. Creosote preserves the stumps, and tissues under the thin creosote layer remain susceptible to infection for several months. If during that time creosoted stumps are damaged, *F. annosus* is able to colonise them.

Because of these and other disadvantages, creosote has been largely replaced by a 10% aqueous solution of sodium nitrite.[4] This is less variable in composition, cleaner to use, and gives better and more consistent control than creosote. It does not prevent all fungi from colonising the stump surface, but allows the entry of some competitors of *F. annosus*. Hence if treated stumps are damaged, or treatment is poorly done, *F. annosus* is less able to enter than is the case following treatment with creosote. In addition to this, sodium nitrite seems to some extent to hinder the growth of any mycelium of *F. annosus* already established in the roots at the time of treatment.

Sodium nitrite is poisonous if swallowed but it is easily handled, and it is not dangerous if proper care is taken, and attention is given to the recommendations for safe use issued by the Ministry of Agriculture, Fisheries and Food.[5] It has now been used in this country for about four years. As the solution is colourless, the dye, disulphine blue, is added to ensure that stumps are neither missed nor incompletely covered.

The contamination of human water supplies must be avoided, and although it would be exceedingly difficult for an animal to lick a toxic amount of the chemical from stumps, it is recommended that unattended domestic animals should be kept out of treated areas for at least two weeks after treatment. Where the use of a toxic chemical cannot be accepted, either the boron compound 'Polybor' (which contains disodium octoborate) or the nitrogenous fertiliser urea may be used. These do not possess all the good properties of sodium nitrite, but they are effective, and safe and easy to handle. 'Polybor' and urea are made up as 10 and 20% solutions respectively, with enough of the dye, Naphthalene red JS, to stain the stump adequately. A better dye than this may be available soon.

Among other promising chemicals now being tested are the herbicides diquat and paraquat.

The quantities of the various stump protectants used per acre depend on local circumstances, but field information shows that creosote was used at about 2 gal/acre, and sodium nitrite has been used over the past few years at about 2·5 lb/acre. 'Polybor' and urea have been used over only a small area, the former at from about 2 to 2·5 and the latter at between 1·5 and 4 lb/acre. In recent years, the Forestry Commission has used chemical stump treatments in over 40,000 acres per year, and the same methods are used in private forests.

Forest nursery disease control

In forest nurseries, there is limited use of fungicides to control a small number of diseases. Two of these, damping-off (caused by various soil fungi, particularly species of *Pythium*) and grey mould (*Botrytis cinerea*) are general ones, with a wide host range, and having importance outside forestry. The others are more specific, with a narrow host range, namely needle-cast of pine (*Lophodermium pinastri*), needle-cast of larch (*Meria laricis*), needle blight of Western red cedar, *Thuja plicata*, caused by *Didymascella thujina*, and mildew of oak (*Microsphaera alphitoides*).

Most damping-off occurs in old nurseries on the heavier soils, especially those that are fairly alkaline. Most newer forest nurseries are on acid heathland, where damping-off is less of a problem.

If damping-off occurs regularly in a nursery, preventive soil fumigation with formaldehyde[6] may be carried out well in advance of sowing, but this treatment is little used. About 10 acres are treated each year in Forestry Commission nurseries, and the treatment is usually given not specifically to control damping-off, but because the plants grow faster in the fumigated soil for reasons still not fully understood.

*Presented at a meeting of the Agriculture Group, 21 November, 1967

If damping-off occurs in the beds of plants, emergency drenches may be used to reduce further loss. Captan is now most often used for this purpose, and again only a few acres a year are treated.

Grey mould (*Botrytis cinerea*) is not a great problem in forest nurseries, though it does sometimes cause damage on Sitka spruce, and is commonly very troublesome in beds of *Sequoia* and cypress.[7]

Various fungicides have been tested against *Botrytis* in forest nurseries. In Italy, some control was obtained in *Eucalyptus* when captan was used.[8] In England, Murray (unpublished) did trials over several years with a number of fungicides, and got the best results with thiram, although phenyl mercury nitrate, griseofulvin and copper oxychloride were also significantly better than the unsprayed controls. Thiram, captan and Bordeaux mixture are all used on a small scale to control grey mould, and the area treated in the Forestry Commission is usually between about 40 and 100 acres per year.

Of the more specific fungi, *Lophodermium pinastri* occurs in Britain on mature trees,[9] especially on Scots pine and to a lesser extent on Corsican pine, but as a source of severe damage it is important only on nursery plants, and then only in occasional years. If *L. pinastri* appears in a nursery, the plants are sprayed with zineb or maneb. The present practice is based mainly on results of experiments carried out in Europe,[10–15] spraying usually being carried out at the end of July, August and September, though it may begin earlier if necessary. In recent years it has been necessary to spray only a few acres, but in seasons in which weather conditions favour the disease, much larger efforts may be needed against it.

Meria laricis mainly affects nursery beds of European larch,[16] and may appear in any part of the country, though usually it is severe enough for spraying to be necessary only in a few areas in Scotland, and sometimes in parts of Wales. At present, needle-cast is controlled by spraying with colloidal or wettable sulphur, the recommendations being based on experiments by Peace.[17] Some recent work in Germany[18] has shown that 0·3% captan and zineb are also effective.

Didymascella thujina (syn. *Keithia thujina*)[19] may build up and cause so much damage in nursery stock that nurserymen are forced to give up growing *Thuja*. Hence, many fungicides have been tested against this disease, but so far only the antibiotic cycloheximide has given any useful measure of control.[20] Cycloheximide has been used successfully for several years on an experimental scale, though so far it has not been generally marketed in this country or cleared under the Pesticides Safety Precautions Scheme of the Ministry of Agriculture, Fisheries and Food.* It has given good results when used at 85 ppm of active ingredient, applied at 100 gal/acre (~1100 l/ha). Usually one application, in late March or April, gives adequate control, though up to three sprayings (in March, April and June) may be needed in some seasons in some wetter western areas.

Finally, fungicides are sometimes used to control *Microsphaera alphitoides*. This disease is very common on oak trees, but is a cause of serious damage only on nursery stock and on young coppice regrowth, and sometimes on young trees not long planted-out in the forest. It may considerably reduce the growth of young plants in their early years. If spraying is necessary to control it, colloidal or wettable sulphur may be used.[21]

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* Since the paper was presented, cycloheximide has been cleared, and marketed as 'Acti-dione Ferrated' (W. Gregory & Co.)

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PHILOSOPHY OF FOREST INSECT CONTROL IN BRITAIN*

By D. BEVAN

It is concluded that, on economic grounds, prevention of infestation by amelioration of the site and improvement in the vigour of the trees is more likely to be practical than remedial spraying of infested trees.

Introduction

Woodlands of all kinds occupy about 7½% of the total land area of Britain. According to the Statement by the Forestry Commission to the 1968 Commonwealth Forestry Conference, the Forestry Commission manages about 1·6 million acres of productive woodlands (of which 1·5 million are under conifers) while private owners manage 1·7 million acres (of which 0·75 million are under conifers). The State plants at the moment about 50,000 and the private owner about 30,000 acres a year. For comparison purposes, the area now designated as arable stands at about 18 million acres and that as permanent pasture at 12 million acres.

It is worth considering typical values per acre under a forest crop. The Annual Report of the Forestry Commissioners for 1966 gives an average price received for wood sold standing as about 1s. 6d. per cubic foot. Taking Sitka spruce as an example, the national average yield expected over the life of the crop is about 140 ft³ per acre per year (the range being ~ 80–280 ft³). The average value of annual timber yield of Sitka spruce per acre is therefore about £10 per acre per year or, if the price at forest gate is taken as 3/-, about £20 per acre per year. From published yield tables, approximate total timber values per acre for the various ages of the example crop can be computed. At 20 years the figure for standing value is about £65 per acre and at 40 years about £230 per acre.

Tree crops are expensive to establish but the investment cannot be recouped until the small plants have grown to a saleable size, and there is a long waiting period; initial costs and all other expenses occurring before harvesting, therefore, are customarily carried forward at compound interest. Early expenses, including those on insect control, because they accumulate interest charges, are unpopular with forest managers. The effect of the long waiting period may be illustrated by noting that, with costs of establishment of the order of £80 per acre, the return on capital by thinnings and final crop sales typically works out at about 3–5%.

Some of the forester's apparent conservatism is based on economic circumstances, owing to the relatively low value of the crop and the long period before profitable yields can be obtained. These circumstances influence every aspect, even the degree to which amelioration of the site before stocking it can be considered. The farmer, having assessed market demand, site potential and the profit to be expected, proceeds to adjust the environment through increased doses of fertilisers, weedkillers, drainage, cultivation etc. to obtain the largest profit. The forester's approach, however, tends traditionally to be the opposite; he makes an ecological evaluation of the site with regard to the tree species it can best support and ensures that his choice grows adequately with the minimum of tending.

* Presented at a meeting of the Agriculture Group, 21 November, 1967

Avoidance and control of infestations

It is not profitable to sustain a forest species through regular protective and ameliorative measures, but it is the poor-growing unhealthy crop which attracts the greatest number of enemies. In long-lived perennial crops there are endemic populations of all organisms but particular species may manifest themselves as pests in one forest and not in another, and it may be concluded that conditions are, for some reason, right for infestation in one forest and not the other. Infestation of some insects can occur for no obvious reason, such pests being referred to as, 'primary'; the defoliators and plant suckers are typical of this group. More explicable are the visitations of the secondary pests; the scolytid bark beetles attack in this way and commonly follow upon damage to, or weakening of, trees through fire, windblow, primary insect attack, or mechanical damage. The control of secondary pests, because their mechanism of infestation is understood, is often a relatively simple matter. Control of primary pests, on the other hand, is often a matter for emergency action, aided by methods of forecasting attack when these exist. For 1966, a total of 825 gallons of insecticide was used by the Forestry Commission. 65% of this was DDT and 32% BHC, the remaining few percent representing a variety of materials for specialised use. The DDT was used for the routine protection of planting stock against the pine weevil, *Hylobius abietis*, and the BHC for the protection of logs against the ambrosia beetle, *Trypodendron lineatum*. Both of these treatments were forms of prophylaxis against secondary pests. The pine weevil seldom fails to breed in the stumps of felled conifers and can 'ring-bark' and kill newly planted replacements. Ambrosia beetle degrades felled conifer timber in the wetter parts of the country if the produce cannot be removed from the site before the beetle's flight period. This is a typical year's usage, but, on the other hand, there are years when some action is called for following outbreaks of a primary pest, such as that taken against the pine looper, *Bupalus piniarius*, at Cannock Chase near Birmingham in 1954 and 1963.

Apart from calamitous introduction of exotic pests, at least some conditions of growth or site are plainly associated with rapid increases of pest populations. For example, if Sitka spruce is planted on a site too dry for it or European larch on a soil too infertile, not only will they both grow poorly but they will be subject to high populations of their respective pests. Thus an incorrect match of species to site can lead to poor growth and susceptibility to attack by pests. Over-age can also lead to increased budworm in Canadian forests.

Poor growth in general might be taken to be a separate predisposing condition, for in spite of the best afforestation techniques available at the time being used, there is now an appreciable acreage of 'checked' and otherwise unthrifty plantations. Such crops are very prone to insect attack. The common factor is tree vigour, and, with indigenous pests at least, if trees can be kept growing vigorously they will

usually suffer relatively little economic damage from their pests.

A great deal of recent research has been done on the use of fertilisers, on new methods and types of drainage, and on the economics of such ameliorative actions in forestry. These developments are primarily concerned with the maintenance of tree vigour but will also have important side-effects on forest pests. There is accumulating evidence, in certain insect species at least, that the physiological condition of the host is the outstanding prerequisite for increases in primary insect pests. This is not to suggest that climate may not act as an overall 'moderator' to insect density, or that natural enemies cannot reduce populations of some species – but only that the host plant has to be in a particular state to support high numbers in the first place.

Conclusions

Approximate calculations on the economics of spraying crops of different species, age and yield class may be made by expressing the response in value terms for every 100 ft^3 of timber volume which protection is likely to save. If it is assumed that the cost of the protection, using aerial spraying, is £5 per acre, with average prices for wood, the treatment is likely to be profitable in a crop of Yield Class 180 if spraying is done when the crop is older than 20 years, but it is profitable only when undertaken after 25–30 years of age in crops of Yield Class 140 and after 35–40 in Yield Class 100. Thus the faster the crop's rate of growth, the later the treatment is given, and the higher the price at which wood is likely to be sold, the more profitable will be the treatment. Therefore the economy of tree crops cannot support anything more than occasional insecticidal treatment, and this only if the crops are reasonably productive. As on the farm one can afford to give luxury treatment to the best crop, not to inferior ones.

It would seem then that the best way to control forest insects is by fertilisers rather than by insecticides, provided that drainage is adequate so that the tree roots are able to make use of the extra nutrient supplied. An important future task for entomologists will be to provide the forest manager with accurate information on timber losses from insect attack. Such losses may not, in themselves justify protective action, but they may contribute to decisions on the returns to be expected from ameliorative measures primarily designed to increase vigour and productivity. Entomologists will also be involved in the study of the mechanisms of host plant susceptibility, and how site amelioration, through host plant physiology, affects the insects' power to reproduce and therefore influences their ecological success, or otherwise, as species.

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USE OF BIPYRIDYL HERBICIDES IN FORESTRY*

By D. F. WILSON

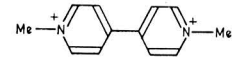
Bipyridylium compounds, particularly paraquat, have been developed as herbicides and cultivation tools in forestry. Uses include weed control in nursery beds and transplant lines, chemical screefing in the forest before planting, weed suppression around young trees, and the maintenance of fire-breaks. Special equipment has been designed to meet these needs.

Introduction

The discovery of the bipyridyliums in the mid-1950s was followed by their evaluation as herbicides mainly in agricultural crops. Investigations on such aspects as structure and activity, mode of action, and metabolic fate in plants, animals and soil, were undertaken mainly in relation to herbaceous crops and the subsequent utilisation of these crops. Developments in forestry came later and, as a consequence of the diversion of most of the research effort on to agricultural crops, the practical application of bipyridyl herbicides in forestry tended to outstrip the more rigorous scientific evaluation of the techniques involved. Valuable contributions from the Forestry Commission did much to redress the balance. The purpose of this paper is to survey the practical experience obtained over the past 10 years or so and to provide the supporting experimental results where they are available.

The bipyridyls are represented in forestry by paraquat (**I**) and diquat (**II**). Their significant properties are: desiccation of all green growth to which they are applied (annual seedling broad-leaved weeds and many grasses are killed outright, perennial broad-leaved weeds may re-grow); rapid absorption by green tissue and therefore reduced removal by rain; safety around trees that have no green bark; and rapid inactivation on contact with soil.

Diquat is active mainly against broad-leaved plants. Paraquat shows greater activity towards grasses but, at the concentration frequently used, is effective against broad-leaved seedlings. A formulated mixture of diquat and



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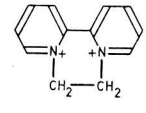

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paraquat is commonly employed where the weed flora contains both broad-leaved plants and grasses.

Scope for weed control in forestry

Weed control problems arise at various stages in forestry operations.[1] They begin with the preparation of land for seedbeds, continue with the maintenance of the beds and the establishment of transplant lines. A major problem often arises in the preparation of an area for afforestation and in the protection of newly planted trees. Some protection from weed competition may be required for up to 5 years. There are also some special problems such as the maintenance of fire-breaks or the removal of shrubs or trees.

Until recent years most of these operations have been undertaken by hand at considerable expense. The main objective in the use of herbicides is either to eliminate hand-weeding, or to reduce the weed flora in the vicinity of the seedlings or young trees so that subsequently hand-weeding is easier and less injurious to the trees or their roots.

Control of weeds in nursery beds

Seeds are either broadcast or drilled in seedbeds and may remain there for one or two years. The seedlings are then transplanted into so-called 'transplant' lines where they are grown on for a further year or two. A fallow may intervene before the land is resown or replanted.

Experiments conducted by Aldhous showed that paraquat could be used on seedbeds of most common conifers as a pre-emergence spray.[2] The sites concerned were mainly Forestry Commission research nurseries but some production nurseries were included in the third year, 1964. The soils, which included many sands with little or no clay content, were acid or very acid.

The results suggested that paraquat was equivalent in effectiveness to vaporising oil. The comparison was based on time taken to hand-weed the beds. Compared with the controls (hand-weeded) both materials greatly reduced the labour requirement. Aldhous specifically investigated phytotoxicity hazards. He showed that paraquat applied post-emergence was lethal to conifers although the response was generally delayed and varied markedly between different species. On the other hand, application about 2–3 days before emergence of the tree seedlings was safe on all except Japanese larch. In this species, the yield of seedlings was reduced, in one out of four trials, by applications of 0·5 to 2 lb paraquat ion/acre.

Lacey, seeking a substitute for hand-weeding (costing up to £100 per acre), reported that seedbeds prepared early could be successfully sprayed prior to seeding with as little as 0·5 lb paraquat/acre.[3] Applications of 0·5–1·0 lb/acre after sowing gave satisfactory weed control and, provided that they were not made less than three days before emergence, caused insignificant phytotoxicity.

The same author also confirmed the suitability of paraquat for weed control in transplant lines when applications were made by special equipment designed to prevent contamination of the young trees.

Control of weeds before afforestation

The preparation of land for planting is an operation subject to severe financial constraints. The common practice is to plough a single furrow for each line of young trees to be planted. Besides giving restricted weed control, this operation may improve drainage or disrupt a hard pan. On the other hand, plough ridges can interfere with timber extraction, and ploughing seems to increase 'windblow', owing to roots tending to run along ridges or furrows. Occasionally, an 18 in planting square is cleared by spade or mattock. This operation, known as screefing, is expensive.

There would thus appear to be a demand for an effective and relatively inexpensive material for 'chemical screefing'. Experience has shown that paraquat serves this purpose when applied in a band 2·5 to 3 ft wide along the planting line, leaving a comparable width unsprayed. The trees are planted in the desiccated bands, leaving the unsprayed strips to give some weather protection.

Despite the obvious technical suitability of paraquat for this chemical screefing in the planting line, it is not as yet widely used. As it is necessary to mark out for screefing, it is then found just as convenient to plant and to spray around the trees with suitable equipment.

Weed killing in young forest plantations

Weed control in young plantations is essential if maximum survival and rapid growth are to be ensured. Traditionally, this has been done by hand. Apart from the expense, this has often meant accidental damage to the young trees and a further cost in replanting.

From 1961 onwards Jack investigated the possibility of using paraquat for weed control in young forest plantations.[4] The preliminary trials gave promising results and in 1963 and 1964 more detailed investigations were undertaken. These were aimed at determining the optimum rates for two different types of application. The results showed that a single application of paraquat with a dribble bar at 1 lb active material per sprayed acre was sufficient to control weeds in young forest plantations for a year.[5] When the 'Arbogard' sprayer (Fig. 1) was used, only 0·75 lb per sprayed acre was required, the material being applied on 4 ft² per tree. Other trials suggested that up to 4500 plants could be treated in one day. Such treatment was likely to be considerably cheaper than hand-weeding in areas where grass growth is vigorous. Current recommendations are for 1·0 lb paraquat per sprayed acre and for an area of 9 ft² per tree.

Aldhous, applying paraquat directly to the bark of conifers in the forest, found that in young plantations many conifers were susceptible to damage.[6] The symptoms may be greatly delayed. The foliage of conifers is killed by paraquat, and many species sustain severe damage if sprayed overall with paraquat during the growing season. After growth has ceased, Sitka spruce, Norway spruce and *Abies grandis* become more resistant and are able to withstand overall sprays of 0·5 lb/acre. In practice, however, the spraying of foliage is not encouraged.

Fire-breaks

The maintenance of fire-breaks is an essential operation in forest management, especially near densely populated areas. The advent of herbicides with a desiccant action prompted the investigation of methods of controlled burning. The objective is the desiccation of the vegetation in the fire-break, followed by burning at a time when the surrounding vegetation is still green and least likely to ignite, thus minimising the danger of a general conflagration.

Connell & Holmes tested paraquat and diquat in 1968 at Coed Morgannwg in South Wales.[7]. Selecting an area of deep *Molinia* grass, they sprayed at 0·25 to 4·0 lb cation in 50 gal water per acre in June, July, August and September.

FIG. 1. *The 'Arbogard' sprayer*

The controlled burning was done 2–3 weeks later. The treatments were effective, paraquat at 0·5 lb/acre being judged the best of those tested. The interval between spraying and burning was not considered critical; burning could be delayed for up to 8 weeks, that is up to the time when the regrowth was beginning to affect the burn. One important advantage of the method was that the small amount of smoke produced was insufficient to interfere with the control of the burning. According to Connell & Holmes, the relatively low cost and the increased ease, speed and safety of controlled burning should reduce costs compared with normal methods. It seemed possible that the technique would permit burning of boundaries that could not be tackled by normal methods.

Paraquat has since come into commercial use for fire-break spraying, the recommended rate being 1 lb/acre in 20 to 60 gallons of water, applied in July or August. It is suggested that to avoid the difficulties associated with regrowth, burning should not be delayed beyond 3 weeks. Much of the development work was done in Australia, where fire hazards are notoriously great.

Other weed control problems

Forests contain many open areas where weed suppression may be obligatory or desirable. Experience has shown that grasses may generally be killed or severely suppressed with paraquat at 1 lb/acre applied through a knapsack sprayer or a tractor-mounted sprayer. The optimum time for application depends on the composition of the sward. Thus the grasses *Festuca ovina*, *F. rubra*, *Holcus* spp., *Lolium perenne* should be sprayed in late autumn, even as late as December; *Agrostis stolonifera* from July to the end of October; *Deschampia caespitosa* in late summer, with care to ensure that spray covers the tussocks and penetrates into them; *Molinea caerulea* in late spring and summer, never later than August.

Ling (*Calluna vulgaris*), cotton grass (*Eriophorum* sp.) and bog myrtle (*Myrica gale*), are suppressed by an application of 1 lb paraquat/acre when the plants are green. Bracken is defoliated by 1–2 lb/acre applied in May or June but recovery is likely in the following year. Gorse (*Ulex* spp.) requires a carefully directed spray (1·5 lb paraquat/acre) in September. Other woody weeds require 2,4,5-T for satisfactory control.

Christmas tree production is an important commercial development in forestry. The close planting demands careful application of paraquat, preferably using a dribble bar. A recommended rate of 1 lb/acre has been derived from experimental work and confirmed in commercial practice.

Application equipment

An applicator has been designed for inter-row weeding with paraquat.[8] A telescopic dribble bar enables the swathe width to be continuously adjusted to cope with variations in row width. The machine is mounted on two small wheels and is propelled by hand. The crop is protected by two large shields, one at each end of the dribble bar. This equipment, known as the 'Xpando' is suitable, without modification, for use in transplant lines. At a forward speed of 2 mile/h it will apply 80–100 gal spray/acre irrespective of row width. Apart from reducing labour requirements, it eliminates mechanical damage to crop roots and provides a safe method of chemical weeding.

For weeding around individual trees in the young plantation, a special machine, the 'Arbogard', has been developed.[8] The problem here is to destroy the weeds in the immediate vicinity of the young tree but to prevent the herbicide from affecting the tree. The machine consists of a plastic guard and a handle to which is attached a gravity-fed pump which forces the spray to a flood-jet nozzle. The spray is distributed over a wide swathe at low pressures, producing coarse droplets and thus minimising drift. Further measures against accidental contamination, include spraying before the weed growth reaches 8 inches and avoiding contact between the operator's clothing and the young trees. Jack reported successful results with paraquat applied through the 'Arbogard' and emphasised the advantages in comparison with hand-weeding.[5] The 'Arbogard' has been in commercial use since 1964. More recently Cooper Pegler and Co. Ltd., have developed the 'Politec' and 'Policones' for use with pressurised knapsack sprayers, which also protect the young trees while the weeds around them are sprayed.

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HERBICIDES IN FORESTRY*

By R. M. BROWN

The present use and future potential of herbicides for control of grass-herbaceous broadleaved weeds, woody weeds, heather and bracken is discussed.

Introduction

Forestry operations in which herbicides are used in Britain can be divided into: nursery work; and plantation work. Because the area under plantations is approximately 1,500–2,000 times as great as that under nurseries, this paper only considers the place of herbicides in plantations.

The Weed Problem

The technical necessity for weeding

It is a well proven fact in forestry that it is necessary to remove weeds to prevent them from competing for light and from toppling on to the young trees at the end of the year, thereby preventing heavy losses which can be incurred subsequently through the effects of pathogenic agencies such as fungi.

Forest weeds include not only grass and herbaceous broadleaved species but woody plants such as bramble, seedling trees, and coppice regrowth. Existing scrub woodland which requires removal before new planting may also be considered as weed growth. Woody weeds compete mainly for light with the crop trees, and are often capable of doing so for many more years than herbaceous weeds because of the height to which they grow. They can also cause considerable damage by rubbing against crop trees during windy periods. There are good practical reasons for their removal, as well as cultural reasons, since they can increase cost of working in plantations by hindering the movement of both man and machine.

In general it has rarely been shown that trees benefit from the removal of the weed competition for nutrients and water in the soil. However, increased growth of crop trees has been reported following the use of herbicides on rendzina soils where trees suffer the competition of a dense grass mat[1] and on acid heathland soils where spruces are in direct competition with heather, *Calluna vulgaris*.[2] Therefore, apart from the possibilities of improved growth in trees on these two types of site, in practice the benefits of weeding appear to be mainly removing both the competition for light and the danger of smothering at the end of the growing season.

Expenditure on weeding

Most of the expenditure on a plantation is incurred in the first five years. During this period the trees are most vulnerable and require assistance to overcome competition from other factors – weeds, animals, insects, fungi, climate. Some help against competitive factors may be required in later years.

At present the Forestry Commission spends about £4 million per year on the direct costs (excluding overheads) of establishing plantations, about 25% of which is spend on weeding.

Private forestry is currently planting about 40 % of the total new plantings in Great Britain. Therefore, the total direct expenditure on weeding in forestry is probably about £2 million per year.

Forest sites show great variation in the vigour of weed growth. Table I shows how weeding costs vary between regions in the Forestry Commission.

In upland Wales and Scotland the ground is usually prepared for planting by deep ploughing. The main object is to improve the drainage and tilth of the soil, but existing weeds are often effectively suppressed for the period during which the crop establishes itself. Sometimes ploughing is undertaken simply as a method of suppressing competing vegetation, but on the generally more fertile sites of England re-invasion is so rapid that further expenditure on weeding is rarely avoided.

Also many sites in the Midlands, South and East England have been occupied by woodland or forest for centuries. A poor cultural history or the need for a change to a faster-growing species frequently necessitates the killing or control of existing woody growth.

Thus it is on the lowland, more fertile sites that the greatest weeding problems occur. There are exceptions, to which reference will be made.

Some basic requirements of forest herbicides

The cost of the herbicide and its application must compete directly with the hitherto traditional methods of weeding by hand and machine. There are three principal requirements:

(a) The ability to control a broad spectrum of weeds – including grasses, herbaceous broadleaved weeds, and woody weeds, e.g., bramble and trees. Not only may these types be mixed on any one site, but the range of species in any group present on the site may be quite large.

Variation between sites is considerable, and reduces the chances of any one herbicide being suitable for wide-scale application.

(b) Potency – one of the biggest differences between agriculture and forestry is the length of time the weeds have been established. Herbicides in forestry are

TABLE I

Cost of establishing Forestry Commission coniferous plantations
Costs, £ per acre (Johnston[3]) exclude cost of land

	Scotland	Midlands, South and East England
Weeding costs	10	45
Total costs	60	120
Weeding expressed as % of total	17	38

* Presented to a Meeting of the Agriculture Group, 21 November 1967

required to control a well established weed flora, which often includes perennials with large and deep roots.

This difference is to some extent mitigated by the fact that absolute clearance of competing weeds from forest crops is not considered necessary, and in fact the presence of a light-scattered weed flora is sometimes considered beneficial because of the protection it affords the trees. Nevertheless, it is the established perennial weeds which often cause the recurring weed problem, and the degree of phytotoxicity required is quite high.

(c) Selectivity – A requirement of any herbicide used in crops. Selectivity is obtainable in ways similar to those in agricultural crops, e.g., crop tolerance, placement, etc. Consideration of the herbicides at present used in forestry will illustrate some of the methods by which selectivity has been obtained.

Major weed types and herbicides

In Table II weeds are classified into five groups which commonly present a weeding problem in forestry, and for which herbicides have been developed.

This Table includes two herbicides, chlorthiamid and dicamba, which are only at the experimental stage. It is hoped that they will illustrate some possible approaches to weed control in forestry not well illustrated by the other herbicides.

Table III shows the quantities of the major herbicides used by the Forestry Commission in the years 1964–66.

TABLE II

Major weed groups, with suitable herbicides

Group description	Herbicides	Status
Mixed weed populations	Mixtures of groups below	rarely used
Grass–herbaceous broadleaved weeds	Paraquat Dalapon Chlorthiamid	widely used small scale trials only
Woody weeds	2,4,5-T Ammonium sulphamate	very widely used moderate scale
Heather and other heaths	2,4-D	moderate scale
Bracken	Dicamba	trials only

TABLE III

Mean annual quantities of the herbicides most used in the Forestry Commission during 1964–66

	Acres treated	Active ingredients, lb
2,4,5-T	5,783	18,725
Paraquat	2,809	2,271
Ammonium sulphamate	858	24,615
2,4,5-T–2,4-D mixture	695	1,805
2,4-D	287	1,032
Dalapon	199	1,883
Total acreage treated	10,631	

Accurate figures of the area on which the use of existing herbicides would provide economic advantages are not available, but assuming that the 13,000 acres planted annually during the years 1964 – 66 in England alone meet this requirement and need weeding for five years, then approximately 65,000 acres would be eligible every year, only about 15% of which are treated with herbicides.

The total Forestry Commission planting programme is about 50,000 acres annually, but much of this is in Wales and Scotland and requires little weeding.

Mixed weed populations

Weed populations are often of a much wider spectrum than the groups into which they have been classified in Table II; for example, grass often occurs as an understorey to bracken. Mixed grass–herbaceous broadleaved weeds frequently contain bramble or other woody weeds. Most herbicides cannot be expected to control such a broad range of weed types and still remain selective to the trees. In fact, no suitable herbicide is available.

For the present, the only possibility of overcoming this problem is to use mixtures of selective herbicides. However, little research has been done on this aspect, and whilst mixtures might appear to be a fairly simple answer, frequently in practice they do not work because the component herbicides have different modes of action or are effective at different times of the year. For example it might seem obvious to try a mixture of paraquat and 2,4,5-T as a foliar application. Unfortunately, paraquat is often most effective on grass–herbaceous broadleaved weeds when applied in October–November or March. As there is no foliage on most woody weeds at this time, 2,4,5-T as an overall spray would be ineffective. Growing-season applications of paraquat would tend to kill the foliage of woody weeds, thus preventing absorption and translocation of 2,4,5-T and an effective kill.

These difficulties have so far been an effective deterrent to the discovery of successful mixtures. Phased applications of the different chemicals is the only current method used for controlling mixed weed populations. Fortunately, one group of the weed flora existing on any site is usually dominant, and in practice adequate weed control is often obtainable by treating this group only.

Mixed grass–herbaceous weeds

Grass is the most important component of this group. Not only is there a suggestion that on some sites grass competition for moisture and nutrients may reduce the growth of the crop, but the aerial portion is often dense and tall and, therefore, extremely damaging. Herbaceous broadleaved plants are occasionally tall and dense, but more often sparse and short, and they rarely produce the dense root mass formed by grass. Thus it is a pre-requisite of any herbicide for this group that it controls most, and preferably all, graminaceous species.

There is no evidence that in Great Britain the commonly occurring broadleaved herbs reduce tree growth because they compete for soil moisture or nutrients. Provided that they are sparse and short they might have a beneficial effect by ameliorating the climate close to the ground.

The herbicide most commonly used to control this group is paraquat, which is toxic to most grasses and effects some control of herbaceous broadleaves. Provided that it is not applied to the foliage or bark of young trees it is completely

selective – there is no soil residue problem. Although paraquat is basically a contact herbicide, under the conditions of lower temperatures and light intensity prevailing in early spring and autumn, it becomes partly translocated. Applications at these times have been found to be more effective than late spring–summer treatments on most grasses, and are capable of providing a full year's control.

Dalapon is the only other herbicide used frequently in practice for this weed group. Since it is ineffective against broadleaved weeds, at rates which do not damage the crop trees, it tends to be used where grass is predominant, for instance on the chalk downlands. Broadleaved weeds may increase rapidly to colonise areas on which grass has been killed, and may create a weeding problem. Dalapon may kill grasses by contact action, but it is mainly absorbed by both root and shoot and translocated within the plant, a property which may enable it to provide effective control of rhizomatous grasses. In spite of this, dalapon has only occupied a minor place as a forest herbicide because there is evidence that applications during the growing season damage the crop, probably because the crop absorbs dalapon from the soil; although applications during September and March are usually safe, and sufficiently toxic to the grasses, this is unpopular with foresters because both months are very busy times of the year – even during these months dalapon sprays should be directed to avoid the tree foliage because unacceptable damage to the crop can be caused; until recently dalapon has been more expensive than paraquat; and dalapon applications are sometimes ineffective because rain shortly before or after application reduces foliar absorption.

Chlorthiamid is still in the experimental stage, but the principles behind its use are interesting since they illustrate another possible approach to weed control.

Chlorthiamid is applied as granules to the ground surface. It is fairly soluble in water and either diffuses or is washed by rain into the upper layers of the soil, where it is quickly broken down into dichlobenil, which is the active agent and which is absorbed by roots.

Chlorthiamid gives good control of a broad spectrum of grasses and herbaceous weeds provided that dry weather does not follow treatment, but it is not certain that at the rates required to do this the tree crop can remain unharmed. Experiments to date have suggested that Sitka spruce, Corsican and Lodgepole pines, oak, beech and sycamore are resistant to applications of 4 lb a.i./acre in March or April.

Some of the damage to other species may be attributable to the difficulty of obtaining an even distribution with granules. Where conditions have permitted the use of tractor-mounted applicators satisfactory distribution has been obtained, but many forest sites are too steep or rocky for tractors, and hand distribution is usually necessary.

Chlorthiamid has been shown to persist in the soil for some months.[4] The evidence suggests that the original kill plus this persistence is sufficient to provide control for the rest of the growing season following application in March or April.

Woody weeds

Examples of woody weeds which often require controlling are bramble (*Rubus fructicosus* agg. L.), regrowth from the stumps of felled broadleaved species, and existing scrub (usually poor quality broadleaved species). The presence of woody weeds at any time during the life of a stand can be undesirable, but it is during the first few years that their control is essential.

Hand-cutting of this type of weed growth can be very expensive – £20 per acre being a not uncommon figure for direct costs. Machine cutting may be cheaper, but like any other form of cutting it does not solve the problem permanently, as many woody species are capable of regrowing so quickly that it is necessary to recut them at frequent intervals.

In the last ten years 2,4,5-T has been used increasingly to control woody weeds, and is beginning to provide remarkable savings in weeding costs. For example it has recently been estimated[5] that the use of herbicides in the South-East Conservancy of the Forestry Commission reduced costs of ground preparation, weeding and cleaning by 20% in 1966. Most of this saving was attributed to the use of 2,4,5-T.

2,4,5-T offers two main methods of control. First, it can be sprayed on to foliage. Application must take place during the growing season when weeds are in leaf, unless the weeds are evergreen or have sufficient area of green stems (e.g. bramble). Fortunately, although they are also evergreen most forest conifers show a marked resistance to overall sprays of 2,4,5-T at the rate required to kill hardwoods provided that applications are made after about mid-August, when the buds are hardening-off, or at any time during their dormant season.

During the conifers' main period of active growth (i.e. May–July) directed sprays of 2,4,5-T can be used provided that it is possible to avoid the trees, and that very high temperatures are not experienced during or following spraying. The latter requirement follows from the ability of 2,4,5-T to volatilise from the treated surfaces during periods of high temperatures, and esters of low volatility should, therefore, be used.

Secondly, applications of 2,4,5-T in a solution of oil (usually diesel oil) to the cut stumps of woody weeds or to the base of standing stems gives effective control of most woody species, provided that stem diameters are not bigger than about 4 in. Larger stems can be killed by similar applications provided that the circumference of the stem is cut at intervals all round (frilled) to facilitate entry of the herbicide. Applications of this type can be at any time of the year, but are usually most effective during the period January to March. The role of oil here appears to be one of aiding penetration into the inner bark and cambial layers of the woody species. Many operators dislike working with diesel oil, and its careless use can be hazardous to the crop.

There are a number of species which have proved resistant to 2,4,5-T and these can usually be killed with ammonium sulphamate. Ammonium sulphamate may be absorbed through the root or leaves, but it is usually applied in water or as crystals to frills or cut stumps.

As a herbicide it has two serious disadvantages compared with 2,4,5-T. It is non-selective, and the ease with which it is washed into the soil and from there absorbed by the tree roots means that it cannot be used amongst crops, or less than about 12 weeks before planting. The second disadvantage is its cost compared with 2,4,5-T.

Heather

This section is concerned primarily with heather (*Calluna vulgaris*), although a number of *Erica* species may occur on the same sites and are controlled by the same herbicide.

As a dominant species, heather occurs on lowland and upland heaths and on many moorlands. On many of these sites weeding costs are low. In fact, the figure of £10 for

weeding in Table I is a reflection of this. However, many of these sites occur in Scotland, Wales and other western districts where spruces are the most successful species, and there is evidence[6] that spruces suffer competition from the roots of *Calluna vulgaris*. Heather frequently provides little aerial competition to the crop trees, but root competition may so reduce the growth of the trees that several years' potential production of timber is lost.

Foliar applications of 2,4-D have been found to give a satisfactory kill of heather, although recent observations in Forestry Commission forests suggest that vigorous healthy heather is more readily killed than sparse leggy heather, which may have insufficient absorptive area.

The difficulties of applying 2,4-D evenly are related to the exposed nature of heathland and moorland sites. Good spraying conditions are rare and some of the poor results may be due to poor distribution.

2,4-D exhibits very similar properties to 2,4,5-T towards the crop trees and selectivity is obtained by the same techniques.

Increases in the growth of crop trees have been reported in a number of cases following kill of heather with 2,4-D.[7,8] Although many of these sites are now ploughed as a preparation for planting, heather often returns quickly because ground conditions are so rocky and vegetation is so matted that complete burial of the heather is rarely attained. There will thus be a continuing need for a herbicide on this type of ground.

Bracken

It has been estimated that there are about 20,000 acres of bracken weeded every year in the Forestry Commission, all of which is now cut manually or by machine.

On the other hand, it is not considered a big problem by foresters because: bracken is *relatively* cheap to cut, even by hand – usually £3 to £4 per acre direct costs for one weeding; and bracken usually grows where trees grow well, and there appears to be no root competition from the bracken.

However, in the first years of a plantation's life it is often necessary to cut the bracken twice a year to prevent it falling on the trees when it dies off at the end of the year.

In experimental work dicamba shows promise for controlling bracken. Indications are that, applied during the period March to May, it can effectively control bracken on most sites for two years, and reduce bracken height and density in the third year.

Dicamba is a hormone weedkiller whose mode of entry is via foliage and roots. The 4th edition of the Weed Control Handbook[9] suggests that the former is more important, although this does not entirely agree with observations that as good a control may be obtained by applying the chemical to 'bare' ground before emergence of the bracken fronds as by applying it subsequent to their emergence.

Although persistence in the soil is only a few months, the long-term control of bracken may be due to the chemical persisting in the bracken litter or rhizomes.[10]

Unfortunately, experiments suggest that applications just prior to planting and post-planting damage trees by root uptake. The interval necessary at the rates required to control bracken is important. If it is too long, a whole year of the two to three years' control of bracken is lost because the trees will have to be planted the following year.

Thus the final assessment on the usefulness of dicamba for bracken control in forestry depends on the interval required between application and planting, the length of effective control, and (not least) the price.

Conclusion

2,4,5-T has made the biggest impact on forest weeding costs of any herbicide. Most other herbicides, particularly those used for control of grass–herbaceous populations have no striking cost advantage over the traditional cutting methods, but are being increasingly used to overcome the shortage of labour in country districts.

If the past trend of herbicide and labour costs continues, the value of developing herbicide techniques will be well justified.

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JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

AUGUST, 1969

1.—AGRICULTURE AND HORTICULTURE

General: Soils and Fertilisers

Age and comparative development of desert soils at the Gardner Spring Radiocarbon Site, New Mexico. L. H. Gile and J. W. Hawley (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 709–716).—The dating of several desert soils by radiocarbon analysis of buried charcoal deposits is presented. A. H. CORNFIELD.

Fundamental objections to the 7th Approximation. R. Webster (*J. Soil Sci.*, 1968, **19**, 354–366).—A critical examination of some fundamental defects of the system is presented and practical implications for soil survey are discussed. A. H. CORNFIELD.

Water-stable aggregates in a forest soil of the West Dehra Dun Division. J. S. P. Yadav and S. P. Banerjee (*J. Indian Soc. Soil Sci.*, 1968, **16**, 31–35).—Water-stable aggregates of different sized fractions occurring at various depths in eight profiles in a *Shorea robusta* forest area are determined. The total amount of such aggregates was highest in the surface layers and decreased with increasing depth in the profile, whereas the proportion of larger aggregates in the total was greater in surface layers and that of smaller aggregates was greater in the lower layers. The amount of org. matter in the soils was positively correlated with the proportion of aggregates > 2 mm. (14 references.) A. G. POLLARD.

Variability of soil chemical properties in two uncultivated Brown Earths. D. F. Ball and W. M. Williams (*J. Soil Sci.*, 1968, **19**, 379–391).—There was considerable spatial variability in certain soil properties in upland grassland sites of unfertilised Brown Earths of apparent uniformity. Coeff. of variation of exchangeable cations (except Na) averaged 33% for 22 samples taken on the same occasion at three locations. Extractable P varied from 29 to 48% at two locations. Variability was high enough to prevent detection of any seasonal trends during a 22-week summer sampling period. A. H. CORNFIELD.

Clay mineralogy of soils of Colombia, South America. G. Mejia, H. Kohnke and J. L. White (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 665–670).—The clay mineralogy of 14 profiles from sea level to 4000 m and varying widely in climatic conditions was studied. A. H. CORNFIELD.

Isotopic distributions of uranium and thorium in soils. R. O. Hansen and P. R. Stout (*Soil Sci.*, 1968, **105**, 44–50).—The concn. of U and Th in granitic rocks and in soils derived from them are examined. The distribution of radioactive isotopes during soil formation processes is also observed. U and Th accumulate during rock weathering; max. concn. of Th normally occur in B horizons and those of U are frequently in surface layers. Downward movement of Th exceeds that of U. Activities of ^{238}U and ^{234}Th are generally similar in rocks, soils and clay fractions. In deeper horizons the activity of ^{230}Th exceeds that of ^{234}U, suggesting the separation of the two isotopes in soil-forming processes. Proportions of nuclides of U and Th are greatest in the clay fractions of soils. (15 references.) A. G. POLLARD.

Distribution of gold in the silt fraction of soil profiles and its genetical significance. N. J. Yassoglou and C. Nobeli (*Proc. Soil Sci. Soc.. Am.*, 1968, **32**, 705–708).—The Au content of various particle size mineral fractions of 13 profiles representing the common genetic soil groups of Greece are presented. Much of the Au was contained in the silt and clay fractions, the amounts ranging from 3 to 120 pt. per 10^9. Parent materials which had undergone one cycle of soil formation contained Au in the silt in resistant metallic form. In other cases Au was associated with easily weatherable minerals. Au is progressively lost from the silt fraction during soil genesis. Au may be used as an index of soil weathering and of the lithological continuity of soil profiles. A. H. CORNFIELD.

Self-diffusion of tritiated water in montmorillonite and kaolinite clay. R. E. Phillips and D. A. Brown (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 302–306).—The self-diffusion coeff. of tritiated water in montmorillonite (*M*) and kaolinite (*K*) increased with the average number of water layers present on each mineral surface. The average number of water layers present on each surface was 4–5 times greater for *K* than for *M*. Self-diffusion coeff. in each clay were approx. equal; the lack of difference between them is discussed and a possible explanation given. (16 references.) A. H. CORNFIELD.

Use of iron-59 for tracing soil particle movement. I. Field studies of splash erosion. II. Laboratory studies of labelling and splash displacement. J. R. H. Coutts, M. F. Kandil, J. Tinsley and (I) J. L. Nowland (*J. Soil Sci.*, 1968, **19**, 311–324; 325–341).—Soil particles were effectively labelled by application of dil. solutions of ^{59}Fe-labelled $FeCl_3$; addition of diethylenetriamine penta-acetic acid (DTPA) improved the uniformity of absorption. The isotope showed little tendency to downward movement by leaching. Even more uniform absorption was obtained by wetting soil peds under vac. Retention of ^{59}Fe increased considerably with decreasing particle size of soil fractions when the whole soil was treated and the clay fraction was dominant in retention of ^{59}Fe. The labelling technique was used to trace the horizontal and vertical movement of soil particles and data for the displacement of soil from bare surfaces by rain splash, as affected by a number of factors, are presented. A. H. CORNFIELD.

Effect of placement of wheat straw on decomposition, evaporation, and soil moisture distribution. P. W. Unger and J. J. Parker, jun. (*Agron. J.*, 1968, **60**, 469–472).—Chopped wheat straw residues (11,000 kg/ha) placed on the surface of a silty clay loam decreased evaporation by 57% compared with mixing the residues with the soil. When the residues were placed in a layer and covered with soil, evaporation was reduced by 19%. Surface residues did not decompose measurably over 16 weeks, whilst mixed and layered residues decomposed at the same rate. Soil moisture content decreased with soil depth below the residue layer for surface and covered residue treatment, whilst with mixed residue, soil moisture increased initially and then decreased with depth. A. H. CORNFIELD.

Hydraulic load-cell lysimeters. T. A. Black, G. W. Thurtell and C. B. Tanner (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 623–629).—The construction, calibration and use of two types of hydraulic load-cell lysimeter (35 metric ton capacity) are described. Daily evaporation from lysimeters and two independent micro-meteorological methods agreed to within 5%. A. H. CORNFIELD.

Electro-osmotic stabilisation of saturated soil systems. G. C. Gupta (*J. Indian Soc. Soil Sci.*, 1968, **16**, 21–29).—The effects of continuous electro-osmosis and the resulting reversal of flow as the clay becomes saturated with Al^{3+} and positively charged are discussed. The reversal of flow occurred even when stainless steel electrodes were used. Data presented demonstrate that under the described conditions the cation exchange capacity of the soil diminishes, and free Al increases in the soil system after the treatment. (11 references.) A. G. POLLARD.

Clay retention, activities, and excised root uptake of ions in bentonite suspensions and dialysates. G. H. Snyder, D. C. Reicosky, E. O. McLean and R. E. Franklin (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 476–480).—Membrane equilibrations of bentonite clay suspensions showed that differences in ion activities between suspension and dialysate phases were greatest at low Ca^{2+} concn. (combined with high K^+ or Rb^+ concn.) and low total electrolyte concn. The 'suspension effect' appears to be predominantly a consequence of dissociation of cations from permanent charges, while hydrolysis of metallic cations from pH-dependent charges tends to mask the effect. A. H. CORNFIELD.

Reduced sodium exchange capacity in unsaturated flow. R. S. Mokady and E. Bresler (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 463–

467).—The effective Na^+ exchange capacity during unsaturated flow through a column of sandy soil decreased as the moisture content decreased. Unsaturated flow may render the soil anisotropic for ion diffusion. The leaching pattern indicated that some of the sites for ion exchange were not readily accessible under unsaturated flow. A. H. CORNFIELD.

Acrylic plastic as a protective coating for easily hydratable and polar-liquid-treated clay minerals. L. P. Wilding, F. P. Miller and L. R. Drees (*Soil Sci.*, 1968, **105**, 2–7).—Samples of some heated vermiculite (*V*) clays tend to become partially rehydrated under ordinary room conditions. The possible coating of particles with a clear plastic (Wizard Aerosol P1802) to prevent this rehydration or, alternatively to prevent loss of ethyleneglycol from glycolated clays is examined. Experimental data showed that rehydration of heated Mg-*V* was not counteracted by the plastic; the org. solvent of the plastic is solvated by the clay and the rehydration proceeds. Ca- and K-*V* appear to be much more stable and rehydrate only slowly. The plastic was much more effective in preventing the loss of org. polar compounds from clay surfaces, e.g., glycolated montmorillonite prepared for *X*-ray analysis, if examined within 6 days. This procedure is less suitable for more precise *X*-ray analysis as the plastic tends to lower peak intensities. A. G. POLLARD.

Alcohol–water interactions on montmorillonite surfaces. II. Ethylene glycol. R. H. Dowdy and M. M. Mortland (*Soil Sci.*, 1968, **105**, 36–43).—The mechanism of glycol retention on homoionic montmorillonite (*M*) surfaces and the effect of adsorbed water on the process are examined. Thin self-supporting films (2 mg/cm^2) of bentonite were prepared as previously described (*Idem*, **15th Conf. Clays and Clay Minerals**, 1967, 259–271). Homoionic Cu, Al and Ca films were saturated with glycol vapour in a vac. desiccator. After 24 h at 115°, the desiccator was cooled to 20° and the films were exposed to the air at 20° and 40% R.H. The wt., *X*-ray and i.r. measurements were made immediately and at intervals as the films were allowed to rehydrate. Cu-, Al- and Ca-*M* were completely dehydrated by equilibration with glycol at 115°. Evidence of cation and org. matter interaction was obtained from the i.r. spectra of glycol–Cu-*M* (bands at 2750 and 2650 cm^{-1}). The bearing of these observations on various aspects of glycol desorption and on theoretical considerations is discussed. A. G. POLLARD.

Cation-exchange equilibrium constants of aluminium-saturated montmorillonite and vermiculite clays. A. E. Foscolos (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 350–354).—Equilibrium const. of Al-saturated montmorillonite (*M*) and vermiculite (*V*) clay minerals were studied in Cl^- solutions at concn. similar to those in soils. The order of relative replacing power in the lyotropic series was, for Wyoming *M* (*CEC* = 90 mequiv./100 g): H < Na < Mg < Ca < K < Al, for California *M* (*CEC* = 125): H < Na < Mg < Ca < Al < K, and for Jeffersite *V* (*CEC* = 175) Mg < Ca < H, < Al < Na. (20 references.) A. H. CORNFIELD.

Exchangeable acidity in unburnt colliery spoil. M. J. Chadwick, S. M. Cornwell and M. E. Palmer (*Nature, Lond.*, 1969, **222**, 161).—Exchangeable acidity was estimated by the $BaCl_2$–triethanolamine method, total cation exchange capacity (*CEC*) by NH_4^+ saturation, and acid-extractable cations (*AC*) with 0·1 N-HCl. For surface spoil (pH 2·8–3·8, 8–50 years old) from three sites, the curve of (exchangeable H^+/*CEC*) *vs. AC* showed a consistent relationship between degree of saturation of ion exchange sites with acidic ions and the potential neutralising capacity (due to presence of ankerite) of the spoil. Where spoil had no neutralising capacity, plant establishment and growth were very poor. Such a curve should aid in identifying sites unlikely to respond to amelioration treatments. W. J. BAKER.

Study of humus metabolism in soils by continuous cultivation. I. Matsura *et al.* (*Microbiology* [*USSR*], 1967, **36**, 584–590).—A suitable apparatus is described and illustrated. (35 references.) P. P. R.

Urease activity in black spruce (*Picea mariana*) humus sterilised by γ-radiation. M. R. Roberge and R. Knowles (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 518–521.)—Urease activity in black spruce litter sterilised by γ-radiation decreased with increase in degree of humus decomposition, and increased when the samples were pre-incubated with addition of urea. Urease activity was directly related to the total and ureolytic numbers of micro-organisms. A. H. CORNFIELD.

Mechanism of soil fungistasis. Wen-hsiung Ko (*Diss. Abstr., B.*, 1967, **27**, 4209).—Of 22 fungi examined, the ability of spores to germinate in soil was positively related to the ability to germinate in absence of exogenous nutrients. Fungi requiring exogenous nutrients for germination did not germinate in soil whereas nutritionally independent fungi generally did so. The failure of some independent fungi to germinate in soil is probably due to the rapid diffusion of nutrients away from the spores into the soil. Spores needing exogenous nutrients did not germinate in aq. extracts of natural soil but did so in extracts from sterilised soil. During the autoclave sterilisation of soil free carbohydrate contents increased 27-fold and amino acids, 37-fold; extracts of the sterilised soil, but not those of the natural soil, contained sufficient nutrients for spore germination. Further evidence indicated that the main bulk of soil, apart from fresh pieces of org. matter, is deficient in nutrients required for spore germination. A. G. POLLARD.

Comparison of methods for isolating soil fungi. C. M. Akhtar (*W. Pakistan J. agric. Res.*, 1968, **6**, 141–153).—A new screened plate method was devised which isolated a greater variety of fungi than did six other methods which were tested. (12 references.) P. S. ARUP.

Study of microbial landscapes in soil as a means of diagnosing soil processes. T. V. Aristovskaya, A. Yu. Daragan and O. M. Parinkina (*Microbiology* [*USSR*], 1967, **36**, 268–271).—An investigation of the soil microbial landscape can give an estimate of the degree of development of the gley process. Soils treated with NH_4^+-N showed an abundance of Fe–Mn bacteria (Gallionella and Siderocapsa) together with an accumulation of ferrous iron; those treated with NO_3^--N contained little Fe^{II} and few Fe–Mn bacteria. (10 references.) P. P. R.

Saprophytic behaviour and survival of *Phytophthora cinnamomi* in soil. S. M. Mircetich (*Diss. Abstr., B.*, 1967, **27**, 3745–3746).—Saprophytism and survival of *P. cinnamomi* in natural soil were examined under controlled conditions, with emphasis on the rôle of chlamydospores in survival. Cardinal temp. affecting germination of the spores were, min. 9–12°, optimum, 18–30° and max. 33–36°. Germination occurred at pH 3–9, but not at pH 2·5. Amino acids (14) and casein hydrolysate induced 72–98% germination of the spores under aseptic conditions compared with only 6% in water. Citric acid, but not glucose, fructose, sucrose, $(NH_4)_2SO_4$ or KNO_3, stimulated germination. The principal factor restricting germination in soil is probably lack of adequate nutrients. Details of the process of germination of spores of *P. cinnamomi* are described and the influence of various soil conditions is discussed. The organism is capable of moderate mycelial growth. Zoospores could move actively up to ~ 50 cm in 10% non-sterile soil extracts and to 5 cm in a saturated, natural soil. A. G. POLLARD.

Growth rates of *Rhizobium trifolii* and *Rhizobium lupini* in sterilised soils. M. S. Chowdhury, K. C. Marshall and C. A. Parker (*Aust. J. agric. Res.*, 1968, **19**, 919–925).—*Rhizobium trifolii* grew faster in a sterilised sandy soil than did *R. lupini* at all temp. up to 30°, the optimum temp. for both species. Temp. > 35° were lethal for both species, the cells of *R. trifolii* being the more sensitive. Growth rates were improved in a fertile sandy loam and in the presence of the appropriate host plants in the sterilised infertile sandy loam. (12 references.) P. S. ARUP.

Characteristics of several strains of pea nodule bacteria isolated from Belorussian soils. T. G. Zimenko, N. M. Gornak and M. K. Kovaleva (*Microbiology*, [*USSR*], 1968, **37**, 273–275).—These were divided, according to their virulence, into various groups. The isoelectric point of the nodule tissues (*NT*) from the inoculated plants was noted and the correctness of the subdivisions was substantiated by vegetative and field experiments. The appearance of pink-coloured nodules together with a pH shift of the *NT* to 4·0–4·8 was regarded as a positive effect. C. V.

Some distribution patterns of hydrocarbon-assimilating microorganisms in oil deposit soils. E. I. Kvasnikov and I. P. Krivitskii (*Microbiology*, [*USSR*], 1968, **37**, 268–272).—Organisms using short C-chains develop more readily in oil-impregnated soil than those preferring paraffins with long chains. Those associated with naphthalene and phenanthrene assimilation would also appear capable of using bicyclic and polycyclic compounds. No indication of species or genus is given. (19 references). C. V.

Soil organic nitrogen mineralisation as affected by low soil water potentials. R. Wetselaar (*Pl. Soil*, 1968, **29**, 9–17).—Two soils of the dry monsoonal region of north-west Australia were incubated (25°) for 3–6 weeks at moisture contents ranging from −2·7 to −50 bar water potential. Nitrate accumulation ceased at −24·3 bar in a clay loam (pH 6·5) and at −50 bar in an alluvial sand (pH 7·2). NH_4^+ accumulated in both soils even at −50 bar. A. H. CORNFIELD.

Effect of partial removal of soil organic nitrogen with sodium pyrophosphate or sulphuric acid solutions on subsequent mineralisation of nitrogen. G. Stanford (*Proc. Soil Sci. Soc. Am.*, 1968, **32,** 679–682).—Removal of increasing amounts of org. N from a loam and a clay loam by extraction with $Na_4P_2O_7$ solutions, varying the concn., temp., pH, and number of extractions, was accompanied by reduction in N mineralisation during subsequent anaerobic incubation. Exhaustive extraction approximately halved the total N content of the soils, but there was still appreciable mineralisation of N during subsequent incubation. Similar results were obtained by hydrolysis with H_2SO_4; the most severe treatment (12–16 h heating with 6 N-H_2SO_4 at 100°) removed about 66% of the total N, but none of the residual org. N mineralised subsequently.
A. H. CORNFIELD.

Effect of topsoil removal on nitrogen-supplying ability of a silty clay loam. H. V. Eck (*Proc. Soil Sci. Soc. Am.*, 1968, **32,** 686–691).—Removal of the surface soil of a silty clay loam to depths ranging from 10 to 41 cm decreased N uptake by sorghum from 41 to 65% under irrigation and from 20 to 62% where moisture was limiting. The effect of removing topsoil was much reduced, especially under limited moisture conditions, by application of N (154–224 kg/ha). Sorghum grain and stover yields were increased to a greater extent by application of N with increasing depth of removal of topsoil. In spite of wide differences in yield due to the treatments, the N content of sorghum tissue was fairly constant (~1% on dry basis). Efficiency of uptake of applied N was not affected by topsoil removal; it remained relatively constant until plant N requirement was met, and then declined. A. H. CORNFIELD.

Available soil-nitrogen index. I. Laboratory and greenhouse studies. II. Field crop evaluation. J. B. D. Robinson (*J. Soil Sci.*, 1968, **19,** 269–279; 280–290).—I. East African soils were conditioned, in order to eliminate the effects of varying periods of air-dry storage, by wetting to 0·33 atm tension., and held at 15–20° for 6 weeks, then air-dried for 3 weeks before being used for tests for mineralisable N. Of a number of methods tested aerobic incubation for 14 days (Bremner, *Methods of Soil Analysis*, Ed. C. A. Black) followed by measurement of the increase in mineral-N ($NH_4^+ + NO_3^- + NO_2^-$) correlated best with tomato yields and N uptake in pot tests.

II. Field trials with maize receiving no N showed that mineralisable N (as determined above) was well correlated with yields and leaf N % in two seasons. Maize yields on plots treated with N were not correlated with mineralisable N in the first season and were correlated only at the lower rate of application (45 kg/ha of N) in the second season. However, maize yield responses to both rates of N (45 and 90 kg/ha) were correlated with mineralisable N in both seasons. A. H. CORNFIELD.

Adsorption of phosphorus by lake sediments. R. D. Harter (*Proc. Soil Sci. Soc. Am.*, 1968, **32,** 514–518).—A portion of the phosphate adsorbed by lake sediment was bonded as Fe or Al phosphates, but some phosphate was adsorbed in loosely bonded form which was independent of the Fe and Al contents of the sediments. Large influxes of phosphate into the lake may be adsorbed temporarily by the sediments and subsequently released to growing plants and algae.
A. H. CORNFIELD.

Anion-exchange resin as a means of assessing soil phosphate status: a laboratory technique. J. Hislop and I. J. Cooke (*Soil Sci.*, 1968, **105,** 8–11).—Finely ground (< 0·5 mm) soil is shaken with the resin (De Acidite, FF 510) and water and the mixture poured on to a Terylene net (~0·5 mm mesh), washed with water and leached with aq. Na_2SO_4 at a controlled rate. The PO_4^{3-} thus removed from the resin is determined by the Fogg and Wilkinson method (*Analyst, Lond.*, 1958, **83,** 406). A. G. POLLARD.

Self-diffusion of phosphorus in clays and soils. II. Effect of pH. G. A. Place, R. E. Phillips and D. A. Brown (*Proc. Soil Sci. Soc. Am.*, 1968, **32,** 657–660).—The self-diffusion coeff. of ^{32}P-labelled PO_4^{3-} in kaolinite, montmorillonite, illite, and silt loam and clay soils was affected by pH, with the greatest differences due to pH being shown by kaolinite. Multiple regression analysis involving self-diffusion of PO_4^{3-} and six soil PO_4^{3-} fractions showed that water-sol. P in kaolinite was positively correlated and occluded Al-P in montmorillonite was negatively correlated with diffusion. When the self-diffusion coeff. and P fractions were averaged over all pH values (3·9–8·7) diffusion increased with increasing water-sol. P and Al-P and with decreasing Fe-P and free oxide content.
A. H. CORNFIELD.

Rate-controlling processes in release of soil phosphate. L. V. Vaidyanathan and O. Talibudeen (*J. Soil Sci.*, 1968, **19,** 342–353).—Isotopic exchange of phosphate in a deep river gravel soil (16% clay), plots of which had been maintained for several years at pH 3·5 to 6·7 and which received no P or 24·6 kg/ha of phosphate-P annually, was governed initially (< 20 h) by simultaneous 'first-order' and 'bulk-diffusion' kinetics only. The 'bulk-' or 'intra-crumb-' diffusion coeff. was independent of phosphate manuring and was at a min. at pH 5·5. The rate const. for the phosphate component exchanging initially with first-order kinetics increased with phosphate manuring at pH 3·5, 4·4, and 5·5, although the const. for unmanured and manured soils did not change with soil pH. At pH 6·7 the rate const. for unmanured and phosphate-treated soils were greater than those in more acid soils, although the const. for the manured soil was just significantly greater than that of the unmanured soil. Residual phosphate (difference between manured and unmanured soils) adsorbed on the soil was greatest in the more acid soils, but water-sol. residual phosphate increased with soil pH to a max. at pH 5·5. Residual phosphate, exchanging initially, decreased to zero above pH 6·5.
A. H. CORNFIELD.

Biological immobilisation of fertiliser phosphorus. I. Accumulation of soil organic phosphorus in coastal plain soils of New Jersey. II. Evaluation of factors involved in phosphorus transformation. A. van Diest (*Pl. Soil*, 1968, **29,** 241–247, 248–256).—I. Comparable uncultivated (usually under deciduous forest) and cultivated soils (under a variety of cropping systems for ~50–200 years) were analysed for total, inorg. and org. P and total org. C and N. Cultivation increased the total, inorg. and org. P in most of the soils, and up to 30% of the increase was accounted for as org. P. Cultivation had nearly always decreased the org. C/org. P and org. N/org. P ratios.

II. Increases in org. P due to cultivation were not due to addition of inorg. P. fertilisers, but to the high quantity and P content, arising from fertiliser treatments, of plant residues returned to the soils. Incubation tests showed that a portion of the org. P contained in crop residues was mineralised slowly. This fraction is probably responsible for the accumulation of org. P in cultivated soils. A. H. CORNFIELD.

Phosphorus retention capacities of some cocoa-growing soils of Ghana and their relationship with soil properties. Yaw Ahenkorah (*Soil Sci.*, 1968, **105,** 24–30).—In 13 latosolic soils of pH 5·3–7·8, the P retention capacities (*PRC*) varied from 4·95 to 15·5 mmol./100 g dry soil. In all soils significant relationships were shown between *PRC* and org. C+Fe, org. C, Fe, pH and clay contents, significance being highest for that with org. C and for the interactions (pH × Fe), (org. C × Fe) and (org. C × pH). In nine soils of pH 6·0–6·5, Fe and the interaction (pH × Fe) were largely responsible for P retention, there being no association between *PRC* and clay or Al in these soils. A. G. POLLARD.

Accuracy of an ignition and an extraction method for measuring organic phosphate in some Canadian soils. R. B. McKercher and G. Anderson (*Soil Sci.*, 1968, **105,** 198–200).—The principal error in the ignition method results from the increased solubility of inorg. P caused by heating; that in extraction methods is largely due to the hydrolysis of org. P during the extraction. The two methods were applied to 60 different soils using the Saunders and Williams ignition method (*J. Soil Sci.*, 1955, **6,** 254) and the extraction method of Mehta *et al.* (*Proc. Soil Sci. Soc. Am.*, 1954, **18,** 443). Evidence obtained indicated, that for surface- and sub-soils the results of the ignition method were considerably higher than those of the extraction method. Descending the profile extraction values fell to very low levels in the deepest horizon, at which depth ignition values were still considerable. The extraction method probably gives an accurate measure of soil org. P in most cases. A. G. POLLARD.

Determination of total phosphorus in soils and parent materials. J. K. Syers, J. D. H. Williams and T. W. Walker (*N.Z. Jl agric. Res.*, 1968, **11,** 757–762).—Fusion with Na_2CO_3 was the most reliable procedure. Digestion with $HClO_4$ gave low results with strongly weathered materials and with samples which contained apatite inclusions; digestion with HF gave slightly low results in samples which contained apatite. Because of occlusion within highly crystalline Fe and Al oxides, secondary inorg. P in strongly weathered soils was less readily extracted by $HClO_4$ digestion than by Na_2CO_3 fusion or HF digestion. (13 references.)
E. G. BRICKELL.

Potassium nutrition of plants in a limed soil. S. C. Mandal and M. K. Sinha (*J. Indian Soc. Soil Sci.*, 1968, **16,** 37–40).—On acidic red loam soil, liming had no consistent effect on the availability of K or on the K nutrition of crops examined. The uptake of K by crops, after liming, was increased in cotton but was

lowered in soyabeans and groundnuts. The Ca uptake was increased in all limed crops. A. G. POLLARD.

Uptake of native potassium by plants from natural soil aggregates of different sizes. M. A. Tabatabai and J. J. Hanway (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 546–549).—Uptake of native K by ryegrass (six cuts) from undried soil aggregates (> 9, 5–9, 3–5, 2–3, and 1–2 mm) was studied in seven surface soils and seven corresponding subsoils. Except for the first two cuts, where dry matter yields and K uptake were slightly higher from the smaller aggregate sizes, aggregate size for each soil had little effect on K uptake or dry matter yields. K uptake and plant K% were highly correlated with exchangeable K (N-NH_4OAc, pH 7·0 extraction) for the soils as a whole, and irrespective of aggregate size or whether top- or subsoils were used. A. H. CORNFIELD.

Effect of plant weathering of soil clays on plant availability of native and added potassium and on clay mineral structure. E. S. Conyers and E. O. McLean (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 341–345).—'Plant weathering' of two soil clays (< 2μ fraction) was produced by growing soyabeans in suspensions of the clays. Uptake of $^{42}K^+$ added to the plant-weathered clays decreased with time of weathering. Initially native K was released from the clays, but with continued weathering fixation of added K^+ ultimately became greater than K^+ release. *X*-ray analysis indicated that plant weathering caused partial degradation of illite and apparent reduction of kaolinite in both soil clays, and reduction of vermiculite in one of the soil clays. A. H. CORNFIELD.

Self-diffusion of sodium ions in frozen Wyoming bentonite–water phase. R. P. Murrmann, P. Hoekstra and R. C. Bialkowski (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 501–506).—The apparent self-diffusion of Na^+ in frozen Na Wyoming bentonite was determined in the temp. range −0·6 to −15°. Although the value of the coeff. decreased with decreasing temp., the migration of Na^+ was relatively high even at the lowest temp. A. H. CORNFIELD.

Manganese uptake attributed to diffusion from soil. E. H. Halstead and S. A. Barber (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 540–542).—The rate of self-diffusion of Mn^{2+}, studied in six soils receiving ^{54}Mn, ranged from $0{\cdot}33\times10^{-7}$ to $2{\cdot}20\times10^{-7}$ cm^2 sec^{-1}. Diffusion rate was correlated positively with soil exchangeable Mn and negatively with pH. Uptake studies with maize seedlings gave a high correlation between Mn uptake and the calculated amount of Mn reaching the root by diffusion. Calculation of the concn. gradient necessary to satisfy the Mn uptake rate by diffusion indicated the Mn concn. at the root surface was reduced only by about 5%. A. H. CORNFIELD.

Insoluble manganese ammonium pyrophosphate found in polyphosphate fertiliser residues. L. R. Hossner and R. W. Blanchar (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 731–733).—When 17 pelleted ammonium polyphosphates (23·4–24·8% P and 5·0–5·8% Mn) were left in contact with a silt loam, 5–80% of the Mn was retained by the pellets after 14 days. There was a high correlation between pH (2·35–4·20) of the fertilisers and the % Mn remaining in the pellets. The Mn compound remaining in the residue was identified as $Mn_3(NH_4)_2(P_2O_7)\cdot2H_2O$. A. H. CORNFIELD.

Effects of adding activated charcoal during dilute acid extraction of manganese from soils. J. W. Gilliam, F. R. Cox, and P. H. Reid (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 511–514).—Addition of activated C (Darco G60) during extraction of soil with 0·05 N-HCl–0·025 N-H_2SO_4) increased the amount of Mn extracted. The extra Mn extracted was not due to release of complexed Mn by the C. The effect was traced to the reducing action of C on Mn^{IV} to produce Mn^{II}; this was confirmed by the fact that extractable Mn levels of a number of soils extracted with dil. acid plus charcoal were similar to those obtained by extraction with dil. acid containing 0·2% of hydroquinone. A. H. CORNFIELD.

Relationship of zinc uptake by maize and sorghum to available soil zinc as measured by three extractants. J. I. Wear and C. E. Evans (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 543–546).—Uptake of Zn by maize and sorghum over 4 weeks in pot tests with 12 soils (medium- to coarse-textured, pH adjusted to about 6·5) was most highly correlated with Zn extracted from the soils by 0·05 N-HCl–0·025 N-H_2SO_4 (r=0·89 for maize and 0·70 for sorghum). 0·1 N-HCl gave r values of 0·82 and 0·63 and 0·05 M-NH_4-EDTA (pH 7·0) gave r values of 0·62 and 0·44 for maize and sorghum respectively. A. H. CORNFIELD.

Interaction of zinc and phosphorus in tops and roots of maize and tomato. K. C. Sharma, B. A. Krantz, A. L. Brown and J. Quick (*Agron. J.*, 1968, **60**, 453–456).—The Zn % in the tops of both maize and tomatoes was reduced by application of $Ca(H_2PO_4)_2$ (25–400 ppm P soil basis) to two clay loams (pH 7·7–7·8) low in extractable P and Zn. The lowest P addition had the greatest effect in reducing top Zn %, the higher additions having progressively smaller effects in this respect. Zn % in the roots was reduced much less by added P. P% in the tops and roots was decreased by the addition of even 1 ppm Zn ($ZnSO_4$) to the soil, the effect being greater where P was also applied. Application of further Zn (up to 25 ppm) had little further effect in decreasing P% in the plants. In presence of added P, addition of Zn increased Zn % more in the roots than in the tops of maize. A. H. CORNFIELD.

Determination of silica in citrate–bicarbonate–dithionite extracts of soils. R. M. Weaver, J. K. Syers and M. L. Jackson (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 497–501).—The colorimetric determination of Si in citrate–bicarbonate–dithionite extracts of soils (for removal of free Fe oxides) is described. Values of Si, Al, and Fe in extracts of soils of different groups are presented and discussed in relation to the formation of montmorillonite from sol. and alluviated materials. (21 references.) A. H. CORNFIELD.

Sulphur deficiency in soils. A world-wide problem. T. W. Walker (*Span*, 1968, **11**, 76–78).—This brief review covers soil and atm. sources of S and the use of superphosphate. E. G. BRICKELL.

Importation of nutrients into woods by rooks. J. S. Weir (*Nature, Lond.*, 1969, **221**, 487–488).—Analysis of faeces and egesta (plant residues plus grit) showed that rooks deposited in Leicestershire woods, below and near the rookeries, a mixture of local rock fragments (granite, sandstone, chalk) and industrial artefacts (brick, basic slag, ceramics, coke, plaster). This grit contributes significantly to the nutrient supply of the wood, viz., Na ~ 6, K ~ 10 and Ca ~ 88 kg/ha during 8 weeks. This input of Ca and K from grit and org. faeces exceeds that from a year's rainfall. Bird populations, even when relatively small, not only introduce org. and inorg. nutrients into woodland, but also cause changes in composition of the total inorg. nutrient input. (10 references.) W. J. BAKER.

Trace elements in a New Zealand serpentine flora. G. L. Lyon, R. R. Brooks, P. J. Peterson and G. W. Butler (*Pl. Soil*, 1968, **29**, 225–240).—The Cu, Ni, Cr, Co, Ca and Mg contents of serpentine soils and nearby non-serpentine soils and of plants growing on these soils were studied. For some species there were high correlations between plant ash and soil contents for Cr, Co, and Ni. *Pimelea suteri* was a strong accumulator of Ni and Co and *Leptospermum scoparium* of Cr. Ca/Mg ratios in the plants were much lower in serpentine than in non-serpentine areas. The use of plant analysis in biogeochemical prospecting is discussed. A. H. CORNFIELD.

Retention of micronutrients by peat humic acids. A. Szalay and M. Szilagyi (*Pl. Soil*, 1968, **29**, 219–224).—Peat humic acid, freed of exchangeable bases, retained Cu^{2+}, Mn^{2+}, MoO_4^{2-}, Zn^{2+} and Co^{2+} virtually quant. when equilibrated with water containing these ions (pH 3 to 5). Deficiency of these nutrients occurring in plants growing on drained moorland or peat soils is discussed. A. H. CORNFIELD.

Reduction of ^{85}Sr uptake in field crops by deep ploughing and sodium carbonate application. R. G. Menzel, H. V. Eck, P. E. James and D. E. Wilkins (*Agron. J.*, 1968, **60**, 499–502).—Deep ploughing (90 cm) reduced the uptake of ^{85}Sr by soyabean, sugar-beet, Sudan grass, and cabbage on a silty clay loam by 50–75%, whilst application of Na_2CO_3 (10 tons per acre) before ploughing reduced uptake by 93–97%. A. H. CORNFIELD.

Effect of beryllium on growth of kale, grass, and mustard. R. J. B. Williams and H. H. Le Riche (*Pl. Soil*, 1968, **29**, 317–326).—The addition of 1–2 ppm Be ($BeSO_4$) to solution cultures and acid soil reduced growth. In calcareous soils addition of even 40 ppm Be did not reduce growth and in one case significantly stimulated growth of kale. A. H. CORNFIELD.

Bronzing disease of rice in Orissa [State] as influenced by soil types and manuring, and its control. B. N. Sahu (*J. Indian Soc. Soil Sci.*, 1964, **16**, 41–54).—In field experiments on a lowland latosol (**I**), a swampy lowland soil (**II**), and on an irrigated bottom land soil (**III**), the effects of various levels of farmyard manure and of N and P fertilisers on the incidence of bronzing disease of rice are examined. The main causes of bronzing were Fe toxicity in **I**, sulphide injury in **II** and Mn toxicity in **III**. Factors affecting the disease in the three soil types are summarised and some symptoms are illustrated in colour. Ameliorative measures included drainage, P and K manuring and liming. Application of easily reducible org. matter to the water-logged soil caused displacement of K from the clay complex and non-availability of K to the plant. Resistance of the

plants to bronzing is lowest in the active tillering stage and application of $(NH_4)_2SO_4$ increases the incidence of disease; foliar sprays with urea have the reverse effect. (31 references.)
A. G. POLLARD.

Residual nitrate in fertilised deep loess-derived soils. G. M. Herron, G. L. Terman, A. F. Dreier and R. A. Olson (*Agron. J.*, 1968, **60**, 477–482).—In silty clay and silt loams appreciable leaching of NO_3^- below the 180 cm depth occurred only where high rates of N were applied. Most of the NO_3^- was found in the surface 90 cm of soil. For a given site there was a high correlation between amounts of NO_3^- in the surface 30 cm and those in the surface 180 cm, although the proportion of the total found in the surface soil varied widely with season. There were high correlations between NO_3^- in the profile in the autumn after harvest and maize yields without applied N in the following year.
A. H. CORNFIELD.

Nitrogenous fertiliser transformations in the Sudan Gezira soil. II. Nitrification of urea and ammonium sulphate. M. M. Musa (*Pl. Soil*, 1968, **29**, 1–8).—Sudan Gezira soil was treated with urea and $(NH_4)_2SO_4$ (200–400 ppm N) and kept in the field in either closed polyethylene bags (to maintain moisture content at 60% of the water-holding capacity) or in open bags to allow normal wetting and drying. The pattern of production of NO_2^- and NO_3^- was similar for both levels of N addition, but differed in magnitude. Urea hydrolysis was arrested during the first week in the open bag system in the summer months. NO_2^- accumulated initially, especially in closed bags, but thereafter fell to low levels. Little NO_3^- was formed during the hot summer months, but there was a gradual NO_3^- accumulation during the winter. Loss of N, probably by volatilisation as NH_3, was on average higher during summer than during winter from both sources of N, was much higher from open than from closed bags, and was somewhat higher from $(NH_4)_2SO_4$ than from urea. A. H. CORNFIELD.

Effect of fertiliser treatments on the chemical composition and yield of maize at different stages of growth. M. Y. Malik, A. A. Sheikh, W. H. Shah and Haleem-ul-Husnain (*W. Pakistan J. agric. Res.*, 1968, **6**, 104–109).—Plots of maize fodder were treated with N and P fertilisers, alone and in combination, and yields were recorded and samples analysed at 30 and 60 days of age. Max. yields and protein contents were obtained with the NP (1:2) combination. The effects of N alone on the protein content of the 30- and 60-day-old plants were small. The P content of the plant was increased in the 30-day-old plants by increasing the P:N ratio, but not in the 60-day-old plants. (15 references.) P. S. ARUP.

Influence of sources of nitrogen, levels of phosphorus and potassium fertilisation on yield and composition of wheat. M. Ali and A. Mian (*W. Pakistan J. agric. Res.*, 1968, **6**, 167–172).—Applications of N, NP and NPK fertilisers gave increased yields above the control in the above ascending order. NK did not produce a better yield than N alone. With or without K, treatments with P and $(NH_4)_2SO_4$ gave slightly better responses than nitrochalk or urea. Greater efficiency was obtained by band treatment than by broadcasting. (16 references.) P. S. ARUP.

Effect of source of phosphorus on its uptake by wheat and clover and on phosphorus fractions in an acid soil. A. N. Smith (*Pl. Soil*, 1968, **29**, 144–155).—When applied at the rate of 50–100 ppm P to a clay loam (pH 4·9), basic slag and $Ca(H_2PO_4)_2$ were more effective in increasing top growth of subterranean clover and plant P % than were rock phosphate (*RP*) and calcined *RP*. Residual effects on a following crop of wheat or clover decreased in the order: basic slag, $Ca(H_2PO_4)_2$, *RP*, calcined *RP*. Results are discussed in relation to changes in P fractions in the soil resulting from application of P materials and cropping.
A. H. CORNFIELD.

Effect of different chemical fertilisers on oil content and fibre characteristics of LS.S. cotton (*G. hirsutum* Lin). M. S. Khan and A. Karim (*W. Pakistan J. agric. Res.*, 1968, **6**, 95–103).—In comparative trials with N, NP, and NPK fertilisers, N alone depressed the oil yield whilst P and K increased it. The lint yield was depressed by N alone, but the fibre strength was increased, especially when N was supplemented by P and K. (10 references.)
P. S. ARUP.

Efficacy of manganese oxide (MnO) as fertiliser for oats. B. van Luit (*Landbouwvoorlichting*, 1969, **26**, 81–84).—In comparative experiments in which Mn was applied at levels up to 50 kg/ha, application of MnO to the soil was less effective for increasing yields than was spraying with $MnSO_4$. P. S. ARUP.

Metal ammonium phosphates: New applications. L. M. Ladina (*Russ. Chem. Revs*, 1968, **37**, 693–701.—The prep. and properties of the double salts of metal and ammonium phosphates is described, particular attention being directed to metal compounds of the composition $MNH_4PO_4 \cdot nH_2O$ which can act as complex, long acting fertilisers. (65 references.) C. V.

Disinfection of soils. Conservatome Recherches (B.P. 1,133,866, 11.5.66. Fr., 17.5.65).—Ionising irradiation (radioactive source or electron beam-generated γ-radiation) is carried out with a wheeled vehicle having the generator screened except for an opening within the wheel base, and fitted with a cut-out safety device. Treatment dose varies from 10,000 to 500,000 rad. J. A. SUGDEN.

Process for improving soil. Henkel & Cie. G.m.b.H. (B.P. 1,136,515, 7.7.67. Ger., 15.7.66).—A process for improving soils chemically and structurally comprises adding a stable, aq. alkaline (pH 7·5–10) SiO_2 sol containing < 6 (0·5–3)% SiO_2, and aq. H_3PO_4 separately (but in either order) to the soil; the final pH of the latter is 5–8 and the final SiO_2 content is 40–200 g/m^2.
S. S. CHISSICK.

Phosphorus fertiliser. Institut Za Naftu (Inventor: V. Logomerac) (B.P. 1,136,319, 25.4.67).—The fertiliser contains a mixture of ground Siemens-Martin furnace slag, from which metallic Fe has been removed, and ground raw phosphate, together with trace elements. One part of ground slag (Fe removed) is leached with 1·5–8 pt. of 10% H_2SO_4 for < 15 min. at $> 100°$ and the mixture filtered. The insol. material containing > 20% of leaching solution is then ground with raw phosphate. S. D. HUGGINS.

Plant Physiology, Nutrition and Biochemistry

Effect of protracted or short-lived application of blue light on photosynthesis in pea plants. N. P. Voskresenskaya, I. S. Oshmarova and Yu. V. Krylov (*Dokl. Akad. Nauk SSSR*, 1968, **182**, 1443–1446).—Pea plants, cultivated in sand at 26°, were subjected to blue (max. 475 nm) or red (max. 650 nm) light for 16 h daily for the first 10–14 days, followed by exposure to white light. The degree of photosynthesis (*DP*) was assessed by i.r. gas analysis. The degree and saturation value of photosynthesis was greater for plants cultivated in blue light. In further experiments, isolated chloroplasts were suspended at pH 6·8 or 7·8 and subjected to blue or red, followed by white light. Photosynthesis was shown to be accompanied by electron transfer, the blue light having a greater effect only when this transfer occurred in conjunction with photophosphorisation. The effect was greater at pH 7·8 or on addition of inorg. P. The effect of initial cultivation in blue or red light, followed by exposure to blue or red (no white light) was investigated; the greatest *DP* was found for plants cultivated in blue light followed by short exposures to red. (13 references.)
J. G. GORODI.

Relation between photorespiration and glycolate oxidase activity in sunflower and red kidney bean leaves. H. Fock and G. Krotkov (*Can. J. Bot.*, 1969, **47**, 237–240).—The results of i.r. gas analysis, CO_2 gas exchange, and manometric determination of glycolate oxidase activity of leaf homogenates showed that the max. rates of photorespiration and glycolate oxidase activity had the same order of magnitude. (15 references.) E. G. BRICKELL.

Driving force on an ion in the absorption process. R. A. Olson (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 660–664).—Data relating to the 'contact effect' controversy were obtained by placing barley roots in suspensions of ion exchange particles and in the corresponding equilibrium dialysate. The rates of absorption of Rb^+ from the two media were not significantly different even though the amount of Rb^+ per unit vol. in the ion exchange particles exceeded that in the dialysate by a factor of $\sim 10^4$. This indicates that the electrochemical gradient rather than the activity gradient is the driving force on an ion in the absorption process. A. H. CORNFIELD.

Concepts of plant nutrient availability in soil systems and nutrient uptake in plants. C. G. Lamm (*J. Indian Soc. Soil Sci.*, 1968, **16**, 9–20).—Theoretical views of the mechanism by which plant nutrients are transported from solid soil particles to the plant are discussed, together with factors influencing the rate at which this transportation takes place. The chief causes of nutrient deficiency are listed. (35 references.) A. G. POLLARD.

Sulphur nutrition of Italian ryegrass in relation to growth and mineral content. A. Ulrich and L. O. Hylton, jun. (*Pl. Soil*, 1968, **29**, 274–284).—Top growth of Italian ryegrass (Tifton No. 1) in

nutrient culture increased rapidly with level of SO_4^{2-} in the nutrient up to 0·125 mequiv. per l but was not increased further by higher levels of SO_4^{2-} (up to 4 mequiv. per l). The SO_4^{2-} content of leaf blade 1 was the most suitable part of the plant for indicating its S status, the critical level for optimum top growth being about 100 ppm SO_4^{2-}-S (dry basis) in leaf 1. A. H. CORNFIELD.

Effect of magnesium and sodium on uptake and distribution of potassium and calcium in cotton. M. W. Thenabadu (*Pl. Soil*, 1968, **29**, 132–143).—Significant Mg–Na interactions were found to govern the uptake and distribution of K and Ca in cotton plants. Under certain conditions addition of Na to the nutrient increased the tissue levels of K and Ca, and this effect of Na was most pronounced when Mg was limiting. A. H. CORNFIELD.

Collapse of lucerne petioles in relation to calcium content. H. I. Nightingale and R. L. Smith (*Agron. J.*, 1968, **60**, 475–477).—When lucerne was grown in nutrient solutions having a wide range of Ca concn. a sudden collapse of the petioles occurred most frequently when shoot Ca content ranged from 11 to 16 mequiv. per 100 g (dry basis). Petiole collapse rarely occurred when shoot Ca content was < 11 mequiv. or > 30 mequiv. per 100 g. Normal petioles showed increasing Ca % as the leaf was approached, whilst the reverse was true for collapsed petioles. A. H. CORNFIELD.

Nitrate uptake by wheat seedlings as affected by calcium and potassium in the nutrient. P. L. Minotti, D. C. Williams and W. A. Jackson (*Proc. Soil Sci. Soc. Am.*, 1968, **32**, 692–698).—In nutrient cultures the uptake of NO_3^- by N-deficient wheat seedlings showed an initial lag phase when Ca was deficient; this lag phase was only partly prevented by the presence of Ca. The subsequent rapid phase of NO_3^- uptake was enhanced when both Ca and K were present, and curtailed by shoot excision and Ca deficiency. More NO_3^- was transported to the shoots where KNO_3 than where $Ca(NO_3)_2$ was supplied, and there was less reduction of absorbed NO_3^- in the shoot with the former salt. A. H. CORNFIELD.

Uptake of phosphorus by plants in relation to carbon dioxide production and organic phosphorus mineralisation in soils. G. S. Sekhon and C. A. Black (*Pl. Soil*, 1968, **29**, 299–304).—In a previous study the total uptake of P by sorghum grown in the glasshouse in 36 soils (pH 7·1–8·3, and including calcareous soils) was correlated significantly with org. P mineralised during laboratory incubation of the soils, independently of labile inorg. P. Further incubation studies, in which CO_2 evolution and org. P mineralised were measured concurrently, showed that total plant uptake of P was correlated significantly with org. P mineralised, independently of correlations with CO_2 evolved and labile inorg. P. However, total P uptake by plants was not correlated significantly with CO_2 evolved, independently of correlations with org. P mineralised and labile inorg. P. These results indicate that mineralisation of org. P is of direct significance in increasing P uptake by plants, the possible indirect effect of CO_2 evolved from soil org. matter increasing the uptake of inorg. P being relatively unimportant. A. H. CORNFIELD.

Effect of light and temperature on nitrate uptake and nitrate reductase activity in rye and oat seedlings. T. M. Chen and S. K. Ries (*Can. J. Bot.*, 1969, **47**, 341–343).—In studies using rye (*Secale cereale* L. cv. MSU Exp. 1) and oat (*Avena sativa* L. cv. Garry) light enhanced NO_3^- uptake and led to the induction of more enzyme, but in plants that received a 16-h dark period before both NO_3^- and dark treatment, no enzyme was induced, although considerable NO_3^- accumulated in the plants. Nitrate and nitrate reductase of oat seedlings, grown at 22° (day) and 17° (night) for 3·5 days, decreased with increasing light intensities. E. G. BRICKELL.

Transport of ^{14}C-labelled assimilates from leaf to apple fruit. J. Wieneke (*Mitt. Klosterneuburg Rebe u. Wein Obstb. u. Früchteverwert.*, 1968, **18**, 462–469).—After assimilation of $^{14}CO_2$ or ^{14}C-sorbitol (**I**) by a leaf on a fruiting apple branch, **I** was the principal sugar (with a little sucrose) to be transported through the bark to the fruit. Within 1 min of $^{14}CO_2$ assimilation, **I** was found to be the principal labelled sugar after which it was transformed into sucrose via glucose and fructose and into polysaccharides and cell wall material. Products assimilated by the fruit itself were also used to build up the fruit material. In the fruit metabolism, malic acid (which could also be transported from the leaves) was the chief intermediate compound. (19 references.) P. S. ARUP.

Effect of inoculation on the exudation of amino acids and sugars by berseem (*Trifolium alexandrinum*) and lucerne (*Medicago sativa*) B. K. Dey, A. N. Sen and W. V. B. Sundara Rao (*Pl. Soil*, 1968, **29**, 213–218).—Aspartic acid was the only amino acid found in root exudates of both inoculated and uninoculated berseem and lucerne, grown in sand culture, 6–9 weeks after sowing. Glutamic acid was found only in the exudates of inoculated plants, whilst asparagine occurred only in the exudates of inoculated berseem. Glucose occurred in the root exudate of inoculated and uninoculated plants of both species after both periods of growth, whilst fructose occurred only in inoculated plants. A. H. CORNFIELD.

Organic acids, sugars, and amino acids during germination of potato. M. M. Milovančev, S. G. Stanimirović and D. L. Stanimirović (*Glasn. hem. Društ., Beogr.*, 1967, **32**, 481–490).—The qual. composition of the tubers, which remained constant, comprised 9 sugars, 5 org. acids, 20 amino acids and starch. Inositol was also found among the sugars, and glucose 1-phosphate among the org. acids. The content of reducing sugars and of sucrose increased during growth as did also the ratio of fructose to glucose (1:1 at the start). The content of galactose increased, and the acidity and starch content decreased. The content of free acids was 4–5 times that of acids combined as salts. (23 references.) (From French summary.) P. S. ARUP.

Transformation of pectins during the ripening of figs. L. G. Semochkina and E. V. Sapozhnikova (*Appl. Biochem. Microbiol., [USSR]*, 1967, **3**, 107–111).—Throughout the growing period, the leaves have a high esterase and polygalacturonase activity. At the commencement of this there is a high to moderate activity of pectolytic enzymes but this ceases upon ripening of the fruit. During the initial stages of fruit development there is a rapid accumulation of pectin (**I**) together with other polysaccharides but in the final stages of ripening the % **I** decreases. During ripening there is an increase in sol. **I** at the expense of proto-**I** and in ripe fruit this constitutes 32–42% of the total **I**. It would appear that proto-**I** is an indispensible part of the cell walls of ripe fruit. C. V.

Physiochemical changes in gluten during germination of wheat. III. Disulphide bonds and sulphydryl groups. O. S. Shorina, A. B. Vakar and V. L. Kretovich (*Appl. Biochem. Microbiol., [USSR]*, 1967, **3**, 287–289).—Amperometric titration of salicylate dispersions of gluten (*G*) showed that the disulphide bonds in *G* decreased during germination while the number of sulphydryl groups increased. The -S-S-/-SH ratio decreased from 63·0 for the original *G* to 30·8 and 19·7 for *G* after germination for 2 and 3 days respectively. One of the causes for this change in *G* during germination is the fission of the disulphide bonds in the protein complex. (16 references.) C. V.

Pipecolic acid as an indicator of abnormal protein metabolism in diseascd plants. G. Palfi and L. Deszi (*Pl. Soil*, 1968, **29**, 285–291).—Pipecolic acid (**I**) was found in the 50%-EtOH extracts of tissue (sampled at flowering) of virus-infected potato, tobacco and soyabean and of rice infected with blast disease (*Piricularia oryzae*), but not in extracts of healthy plants. **I** was also found in the leaves of rice sprayed with maleic hydrazide, but not in control leaves. A. H. CORNFIELD.

Utilisation of ^{15}N and phenylalanine-2-^{14}C by wheat plants. A. J. Finlayson and W. B. McConnell (*Can. J. Biochem.*, 1969, **47**, 415–418).—The location of ^{15}N in the upper parts of the wheat plant after injection of $^{15}NH_4Cl$ into the stems at intervals during kernel formation was markedly dependent on the time of tracer injection, and the results suggested that the gluten proteins and the salt-sol. proteins were formed independently of each other. The rate of incorporation of phenylalanine into protein, relative to its metabolism by other pathways, increased as the plant matured. E. G. BRICKELL.

Metabolism of ^{14}C-labelled proline in higher plants. D. Wang (*Contr. Boyce Thompson Inst. Pl. Res.*, 1968, **24**, 117–122).—Maize leaves rapidly converted proline-5-^{14}C to glutamic and aspartic acids and kinetic and radioactive data showed that the synthesis occurred via the proposed reaction sequence for micro-organisms and animals. Alanine was the only other amino acid to be labelled (slightly) and there was no detectable amount of isotope in any of the acids, such as hydroxyproline, ornithine, citrulline and arginine, which are metabolically closely related to proline. No ^{14}C was incorporated into glutamic or aspartic amides. E. G. BRICKELL.

Metabolism of amino acids and amides in germinating seeds. D. Wang (*Contr. Boyce Thompson Inst. Pl. Res.*, 1968, **24**, 109–115).—There was a sharp decline in asparagine accompanied by a rapid increase in glutamine in wheat during germination. With further development, a shift from a glutamine-predominant phase to an asparagine-predominant phase took place and the content of

asparagine amounted to >80% of the total free amino acids and acid amides within 72 h following germination. On the other hand, proline in maize seedlings decreased with a concomitant increase in glutamine and asparagine, showing that a metabolism shift from a proline- to an amide-predominant phase occurred. A close correlation existed between the ratio of glutamine to asparagine and the physiological age of tissue or seedling growth. (23 references.)
E. G. BRICKELL.

Nucleic acids and ribonucleases of wheat leaves and chloroplasts. D. Hadziyev, S. L. Mehta and S. Zalik (*Can. J. Biochem.*, 1969, **47**, 273–282).—High ribonuclease B activity associated with chloroplasts is localised in the stroma and the chloroplast membrane, and a negligible amount is associated with mitochondria and ribosomes. Its retention is affected by the isolation medium used and its activity is enhanced by cold treatment. Ribonuclease A, which is confined to the cytoplasm, is removed only to the extent of 50% by bentonite, part being bound as an inactive complex. The methylated albumin kieselguhr elution profiles of nucleic acids are affected by ribonucleases, mainly the ribosomal types of RNA being degraded, particularly for chloroplasts from older leaves.
E. G. BRICKELL.

Relationship of vegetable colour to physical state of the carotenes. A. E. Purcell, W. M. Walter, jun. and W. T. Thompkins (*J. agric. Fd Chem.*, 1969, **17**, 41–42).—The absorption spectra of fresh and heated carrots, sweet-potato, squash and tomato were compared. Heating caused very little alteration in the spectra of aq. suspensions, but, after boiling these for 2 min, the max. for carotene broadened and flattened, although the main absorption remained in the same regions. The change is probably due to the degradation of the chromoplasts and solution of the carotenes in other cellular lipids.
P. S. ARUP.

Properties of root-nodule bacteria from a single vetch nodule. M. Z. Machavariani (*Microbiology* [*USSR*], 1967, **36**, 591–594).—Several strains of *Rhizobia*, differing in their behaviour towards carbohydrates and amino acids, were isolated from a single nodule. They differed in dehydrogenase activity and in sensitivity to antagonistic actinomycetes.
P. P. R.

Determination of nitrogen fractions in plant material. O. Carpena, S. Navarro and E. Hellín (*Revta Agroquim. Tecnol. Aliment.*, 1968, **8**, 360–366).—Modified methods for the routine extraction and determination of total-, protein-, chlorophyll- and total soluble non-protein-N in plant materials are described. Coeff. of variation obtained in the analysis of standards (NH_4NO_3, glutamine, asparagine and simple amino acids), and of physiologically normal foliar material from several species of citrus, are tabulated. (16 references.)
E. C. APLING.

Relationship between chemical constitution of organic compounds and their biological activity. M. Kirigin, D. Kolbah and E. Cerkovnikov (*Bull. scient. Cons. Acad RSF Yougosl.*, Section *A*, 1969, **14**, 7–9).—The prep. of 3-(1′-oxacyclohexanyl-4′)-2-oxapropane carboxylic acid-1 [(tetrahydropyran-4-yl)methoxyacetic acid] (**I**) is described. The phytohormonic activities of **I** and 4-chloro-2-methylphenoxyacetic acid (**II**) were compared at levels between 0 and 1·0 μg by measuring the root growth of soyabean. The results show that **I** is a plant hormone and that it gives less pronounced inhibition of growth than the well known hormone **II**.
W. E. ALLSEBROOK.

Isomerism in quaternary ammonium derivatives of (+)-limonene and its effect on plant growth. W. F. Newhall and A. P. Pieringer (*J. agric. Fd Chem.*, 1969, **17**, 153–155).—Tests on bean plants (growth retardation) showed that racemic limonene can be used just as well as the optically active isomers for the prep. of quaternary ammonium alcohol deriv. of *p*-menthane.
P. S. ARUP.

Effect of growth stimulants and inhibitors on root formation in lemon cuttings. T. V. Nekrasova and M. Kh. Chailakhyan (*Dokl. Akad. Nauk SSSR*, 1968, **182**, 221–224).—The effects on root development of the following compounds were studied singly or in combination:—indole-3-acetic acid (**I**), indole-3-butyric acid (**II**), gibberellic acid (**III**), maleic hydrazide (**IV**), chlorocholine chloride (**V**). The cuttings (top or lower ends) were immersed for 22–24 h in the solution, then the cuttings were transferred to a sand seedbed and plant development observed for 50 days at 18–22°. **I** and **II** stimulated root growth, **III** and **IV** retarded it. **V** aided the stimulating effect on root development of **I** and **II**, while **III** and **IV** reduced the effect of **I** and **II**.
J. G. GORODI.

Induced femaleness in cucumber by 2-chloroethanephosphonic acid. S. Iwahori, J. M. Lyons and W. L. Sims. (*Nature, Lond.*, 1969, **222**, 271–272).—Two applications (at first and second leaf stage) of 20 μg of 2-chloroethanephosphonic acid (**I**) to monoecious cucumber plants retarded growth, decreased time to anthesis of first female flower, and increased the no. of female flowers. One application (100 or 250 ppm) of **I** at first leaf stage to glasshouse-grown monoecious and field-grown gynoecious cucumber plants induced complete femaleness. Treatment of intact plants with **I** as a substitute for ethylene is discussed, mainly in respect of sex regulation in cucumber by the endogenous gibberellin–auxin balance.
W. J. BAKER.

Factors influencing the genetics of reaction of barley to root rot caused by *Helminthosporium sativum*. E. Cohen, S. B. Helgason and W. C. McDonald (*Can. J. Bot.*, 1969, **47**, 429–443).—The inheritance of seedling reaction to root rot caused by an isolate of *H. sativum* was studied in crosses and backcrosses among two varieties of barley which showed resistance and one which was susceptible. Polygenic inheritance for seedling reaction was indicated as being influenced by seed wt. Genotypic inheritance was not established conclusively. Field studies showed that there was no correlation between disease indices of cabinet-grown seedlings and their reaction in the field, only one of the resistant species being susceptible. (39 references.)
C. J. R.

Adult plant leaf rust resistance in Thatcher and Marquis wheat: a genetic analysis of the host-parasite interaction. P. Bartos, P. L. Dyck and D. J. Samborski (*Can. J. Bot.*, 1969, **47**, 267–269).—Inheritance of adult plant resistance to race 9 of leaf rust was investigated. Resistance was conferred by the same recessive gene in both varieties. A single recessive gene conferred virulence on adult plants of Thatcher; this gene was inherited independently of the genes that condition virulence on host genes *Lr1*, *Lr2*, *Lr3* and *Lr11*.
E. G. BRICKELL.

Controlling plant growth. Fujisawa Pharmaceutical Co. Ltd. (B.P. 1,133,902, 5.4.67. Jap., 7.4.66).—*m*-Trifluoromethylphenoxyacetic acid and its org. and inorg. salts and esters are claimed as active constituents of compositions for controlling height growth of Gramineae plants, and make up 1–80 (10–50)% by wt. of a powder, solution, etc. Examples describe their use on rice, Italian ryegrass, etc.
S. S. CHISSICK.

Stimulation of metabolic function of macroscopic plants. Crown Zellerbach Corp. (Inventor: R. J. Herschler) (B.P. 1,134,780, 21.3.67).—Dimethyl sulphoxide (**I**) is claimed as the active constituent of mixtures useful for stimulating plants, resulting in an increased rate of growth and/or an increase in products (e.g., wax, rosin, oil) and/or an increase in fruits. An aq. solution containing >25% of **I** together with (if required) an antifreeze (e.g., acetone) is sprayed or painted on the plant or injected into the xylem at intervals of 14–28 days during the growing season.
S. S. CHISSICK.

Crops and Cropping

Plant breeding for disease resistance. H. C. Smith (*Span*, 1968, **11**, 89–91).—The merits of breeding for 'specific' and for 'general' resistance (i.e., resistance to physiological races of pathogens and to the entire pathogenic spectrum) are discussed, the latter policy being favoured.
E. G. BRICKELL.

Occurrence of octadeca-*trans*-2,*cis*-9,*cis*-12-trienoic acid in pollen attractive to the honey bee. C. Y. Hopkins, A. W. Jevans and R. Boch (*Can. J. Biochem.*, 1969, **47**, 433–436).—This new unsaturated trienoic acid was isolated from mixed pollen gathered by honey bees and was identified. The acid was shown to be an attractant for the honey bee. It is produced by the plant and acts as a food marker for the insect. (15 references.).
E. G. BRICKELL.

Presowing drought hardening of wheat. D. R. Woodruff (*Aust. J. agric. Res.*, 1969, **20**, 13–24).—Glasshouse and field experiments are reported. In general, the treatment reduced the rate at which critical levels of relative water content (*RWC*) developed during periods of moisture stress, but not necessarily the final value to which the *RWC* fell. Increases in grain yield due to the hardening treatment varied from 0 to 20% in the different experiments due to the phenological stage at which the *RWC* differences occurred and their transient effect. (20 references.)
E. G. BRICKELL.

Effect of gamma irradiation on yield and its components in wheat. S. S. Shah, M. H. Saleemi and G. A. Shah (*W. Pakistan J. agric. Res.*, 1968, **6**, 10–18).—Dry seeds of seven wheat varieties were subjected to 20–30 kr doses of γ-radiation, and the effects on no. of spikes per plant, no. of spikelets per spike, no. of kernels per spike and spikelet, 100 kernel wt. and yield per plant were studied

in M_1 and M_2 generations. In general, the irradiation had a depressant effect, the magnitude of which varied with the dose strength. Most of the effect was restricted to the M_1 generation. (16 references.) P. C. W.

Effects of ammonium phosphates containing varying nitrogen: phosphorus atom ratios on emergence of wheat. C. K. Stevenson and T. E. Bates (*Agron. J.*, 1968, **60**, 493–495).—Ammonium phosphates having atom ratios of N/P ranging from 1/1 to 2/1 [$NH_4H_2PO_4$, $(NH_4)_2HPO_4$ and their mixtures] were applied in solution in the row with wheat seed at planting in soil (pH 5·4 to 7·4). Soil pH had no effect on emergence with any of the materials tested. In the clay loam neither early nor final emergence were affected by varying the N/P atom ratio when the materials were applied at the same P rate. In the sandy loam both early emergence and, in particular, final emergence were markedly lower with the high than with the low N/P ratio materials. A. H. CORNFIELD.

Grain yield and kernel quality of winter barley harvested at different moisture levels. D. H. Brewer and J. M. Poehlman (*Agron. J.*, 1968, **60**, 472–474).—When winter barley was harvested, beginning with grain moisture content of ~ 50% and continuing until it decreased to ~ 10%, grain yield, 1000-kernel wt. and test wt. increased as moisture content declined to ~ 40%, but remained relatively constant with further decreasing moisture content. Kernel size increased with decreasing grain moisture up to ~ 30% and then remained relatively constant. Barley colour and N % were not significantly affected by moisture content at harvest. A. H. CORNFIELD.

Effects of lime and phosphorus treatments in specific horizons of an acid soil on growth of maize. J. Estrada and G. A. Cummings (*Agron. J.*, 1968, **60**, 447–450).—Application of superphosphate (90–360 kg P per ha) to the Ap horizon (pH 4·9) of a loamy sand increased maize top growth to a greater extent than did application to the A2 horizon (pH 5·1). Application of CaO (2074 kg per ha) to the A2 horizon increased root growth in that horizon and this was not increased further by CaO application to the Ap horizon. Max. top growth was obtained when CaO was applied to the Ap horizon. Liming decreased foliage P and Al (%). Foliage Al % was decreased to a greater extent by P than by CaO application. A. H. CORNFIELD.

Plant constituents of an early and a late maize hybrid as affected by row spacing and plant population. H. T. Bryant and R. E. Blaser (*Agron. J.*, 1968, **60**, 557–559).—Stalks, leaves and husks comprised a larger and ears a smaller proportion of the total dry wt. of the late than of the early hybrid. Comparing row spacings of 36 to 89 cm the proportion of stalks to total dry wt. of both hybrids was least from rows 53 cm apart. Total top wt. increased slightly with increase in distance between rows. The wt. of the individual plant constituents, averaged for both hybrids at all row spacings, decreased with increasing plant population (39,500 to 98,800 plants per ha). Average yield of silage was larger, but average yield of grain was smaller, from the late than from the early hybrid. Silage yields from both hybrids were highest from the highest plant population. A. H. CORNFIELD.

Maize yield response and economic optima for nitrogen treatments and plant population over a seven-year period. D. Colyer and E. M. Kroth (*Agron. J.*, 1968, **60**, 524–529).—Max. and economic optimal treatments and yields of maize were studied over 7 years on two silt loams receiving N ranging from 28 to 224 kg per ha and with plant population ranging from 2×10^4 to 5×10^4 per ha. There were considerable differences in optimal treatments from year to year and between the two sites. Over the 7-year period the economically optimal treatment was 120 kg N and 37,787 plants per ha. A. H. CORNFIELD.

Boron deficiency in maize. K. W. Smilde and B. van Luit (*Landbouwvoorlichting*, 1969, **26**, 78–80, 84).—B deficiency causes imperfect fertilisation with greatly reduced grain production, and is most likely to occur on sandy soils containing < 0·3 ppm of B, during dry seasons. The deficiency can be remedied by the application of borax at 10 kg/ha. P. S. ARUP.

Water relations of maize. I. Effect of severe soil moisture stress imposed at different stages of growth on grain yields of maize. II. Effects of nine irrigation regimes on maize. J. H. Wilson (*Rhod. J. agric. Res.*, 1968, **6**, 103–105; 107–108).—I. Severe soil moisture stress imposed on the plants during 8 days (in large polyethylene bags) during grain filling caused leaf senescence, or, when imposed between the late vegetative and early grain development periods, reduced the no. of grains per plant by up to 32%. Drought just after flowering reduced grain filling so that yields fell by up to 49%. Two or more consecutive droughts depressed yields by up to 66%.

II. Droughts were imposed on wet, medium, and dry soil regimes. Drought before flowering delayed tasselling and silking, and reduced the grain no. Irrigation just before flowering was most effective in compensating, by increased grain wt., for harm done during any of the previous regimes. Leaf senescence was promoted by drought during grain ripening, but prevented by waterings before or after flowering. P. S. ARUP.

Dry matter production and recovery of fertiliser nitrogen by rice as affected by nitrification retarders. R. Prasad (*Pl. Soil*, 1968, **29**, 327–330).—Three successive crops of rice seedlings were grown for 15 days each in a clay loam (pH 8·2) treated initially with 100 ppm N as $(NH_4)_2SO_4$ or $NaNO_3$. In addition, 0·5–2·0 ppm of N-Serve [2-chloro-6-(trichloromethyl)pyridine] and 1–5 ppm of AM (2-amino-4-chloro-6-methyl pyrimidine) were added to some of the $(NH_4)_2SO_4$-treated pots. Dry matter yields were higher where $(NH_4)_2SO_4$ than where $NaNO_3$ was applied, and were increased a further 5–11% by addition of the retarders to the $(NH_4)_2SO_4$-treated soils. Recovery of applied N by the rice tops was 8% where $NaNO_3$ and 37% where $(NH_4)_2SO_4$ was applied. Recovery increased to 53% where N-Serve and 45–48% where AM was applied together with $(NH_4)_2SO_4$. A. H. CORNFIELD.

Timing of nitrogen fertilisation of rice. II. Culm elongation as a guide to optimum of applications near midseason. V. L. Hall, J. L. Sims and T. H. Johnston (*Agron. J.*, 1968, **60**, 450–453).—The relationship between grain yields and time of application of N (45–135 kg per ha) around midseason was studied, particularly in relation to the length of the main internode below the panicle. Top-dressing of N was most effective in increasing grain yields of three varieties of rice when 50% of the plants had internodes < 12·8 mm for the variety Vegold, 39·7 mm for Nato, and 32·1 mm for Bluebonnet 50. A. H. CORNFIELD.

Effect of row spacing and seeding rate on forage production and chemical composition of two sorghum cultivars harvested at two cutting frequencies. H. R. Koller and J. M. Scholl (*Agron. J.*, 1968, **60**, 456–459).—Herbage yields and lignin % were higher and forage N % was lower when sorghum was cut twice rather than 3 times during the season. Forage yields from 17·8- and 35·6-cm rows were similar and exceeded those from 71·1-cm rows. Row spacing had little effect on chemical composition. Forage production increased with sowing rate (8·4–33·6 kg per ha in one year and 13·5–53·8 kg per ha in another year), particularly in narrow rows. Forage N % decreased and lignin % increased at the first harvest as seeding rate increased, but only when cut 3 times per season. A. H. CORNFIELD.

Control of manganese deficiency in potatoes with one application for yield increase. C. Mulder and N. P. Borst (*Landbouwvoorlichting*, 1969, **26**, 85–90).—Incipient deficiency symptoms are a lightening in leaf colour and yellowing; at this stage, and well before the appearance of brown stripes, spraying with $MnSO_4$ at 15 kg/ha has a distinct effect in reducing the deficiency and increasing the yield. Spraying with $MnSO_4$ is more effective than dressing with MnO. A single spraying is generally sufficiently effective. P. S. ARUP.

Growing table beets. V. R. Boswell (*Leafl. U.S. Dep. Agric.*, 1968, No. 360, 4 pp.).—Soils and fertilisers, varieties, planting and culture, harvesting, handling and storage are discussed. E. G. BRICKELL.

Changes in composition of Sudan grass and forage sorghum with maturity. M. B. Farhoomand and W. F. Wedin (*Agron. J.*, 1968, **60**, 459–463).—The % of dry matter, crude protein and crude fibre in the leaves, stems and heads of Sudan grass and forage sorghum cut once after a killing frost, cut once in summer and cut three times in summer are reported. A. H. CORNFIELD.

Effect of nitrogen fertiliser on an old stand of crested wheatgrass, *Agropyron desertorum*. W. J. MacGinnies (*Agron. J.*, 1968, **60**, 560–562).—When urea was applied at 22 to 112 kg N per ha annually to a 16-year-old stand of crested wheatgrass, herbage yields increased with rate of N, although increase in yields per kg of N applied declined with increasing rate of N. Over 6 years annual applications produced higher yields than did biennial applications. Single applications of 22–45 kg N per ha increased herbage yields in the first year, 67–225 kg N increased yields in the 4 years following, and 449 kg N increased yields in the 5 years following application. A. H. CORNFIELD.

Effect of fertilisers and soil texture on accumulation of nitrate and other nutrients in two varieties of Bermuda grass, *Cynodon dactylon*.

D. A. Lovelace, E. C. Holt and W. B. Anderson (*Agron. J.*, 1968, **60,** 551–554).—Irrespective of fertiliser treatment the forage of NK-37 Bermuda grass contained approx. twice as much NO_3^- as did that of Coastal Bermuda grass. Forage NO_3^- level in a sandy loam was higher than in a clay loam in the first harvest, but the reverse was true in the second harvest. Rate of applied N [67 or 336 kg per ha as $Ca(NO_3)_2$] had little effect on forage NO_3^- level, except in the second harvest from the clay loam, where it was higher with the higher level of applied N. Application of other major and trace elements did not affect forage NO_3^- levels.

A. H. CORNFIELD.

Effect of burning on Coastal Bermuda grass forage production. H. D. Morris (*Agron. J.*, 1968, **60,** 518–521).—Burning Bermuda grass pasture in Jan., March or April increased subsequent forage yields, but only where high NPK was applied. The beneficial effect of burning was attained in all 3 years at the second clipping (July). Burning provided more effective weed control than did mowing or herbicide applied in mid-April, and late burning was more effective than early burning. After 3 years there was no difference in the total N content of soils between burned and unburned plots. A. H. CORNFIELD.

Long-term fertility requirements of Coastal Bermuda grass I. Potassium. W. W. Woodhouse, jun. (*Agron. J.*, 1968, **60,** 508–512).—The performance of Coastal Bermuda grass on a sandy soil receiving adequate N and P and varying levels of K was studied over an 11-year period. Annual applications of 46·5 kg K per ha increased forage yields by about 33% in the first year and up to 100% in the following years. After 7 years there was practically no stand where no K had been applied. Applications of 93 or 186 kg K per ha gave only moderately increased yields compared with 46·5 kg K. Application of K at the low and medium rates enabled the grass to remove far more K from the native supply than was removed under the no-K treatment, measured over the 11 years. Forage K % increased annually where the highest K rate was applied, but remained essentially the same with the other rates. Although forage K % varied widely, there was a significant trend for it to decrease from the beginning to the end of the season. Roots penetrated to a depth of at least 244 cm.

A. H. CORNFIELD.

Breeding tropical pasture plants. E. M. Hutton (*Span*, 1968, **11,** 72–75).—A review with particular reference to Australia and areas south of the Tropic of Capricorn. Aspects covered include breeding objectives, increased dry matter and protein yields, increased persistence, resistance to diseases and pests, improved nodulating activity and increased seed production, for legumes, competitive ability of legumes with grasses, and feeding value, persistence and drought and frost tolerances, for grasses. (10 references.) E. G. BRICKELL.

Electronic instrument for pasture yield estimation. I. General relationships. H. L. Black (*J. Br. Grassld Soc.*, 1968, **23,** 216–222).—An electronic instrument (*ibid.*, 1962, **17,** 89) based on measuring the frequency of an oscillator circuit in a probe placed in the herbage, was tested for measuring pasture yield. Much field work comparing instrument readings with dry matter yields indicated that no general relationship of acceptable accuracy has yet been found. Further work using a double-sampling technique is in progress. A. H. CORNFIELD.

Microwave drying of herbage. D. I. H. Jones and G. ap Griffith (*J. Br. Grassld Soc.*, 1968, **23,** 202–205).—Microwave heating (radio-frequency dielectric heating) was able to dry 400 g of fresh herbage in 15–20 min. Drying by microwaves or freezing produced herbage with similar water-sol. carbohydrate contents. Microwave drying often produced herbage of a higher water-sol. carbohydrate content than did forced-air drying. A. H. CORNFIELD.

Yield response prediction and manganese soil test interpretation for soyabeans. F. R. Cox (*Agron. J.*, 1968, **60,** 521–524).—Yield responses of soyabeans to Mn applications were obtained when plants on untreated plots contained < 20 ppm Mn in the uppermost fully developed leaf or in the mature seed. The degree of response could be predicted from soil pH and the level of soil Mn as determined by extraction with 0·050 N-HCl–0·025 N-H_2SO_4. The level of extractable Mn required for normal growth was greater at high than at low soil pH. A. H. CORNFIELD.

Effects of boron and manganese on cotton yield, lint quality, and earliness of harvest. O. E. Anderson and F. C. Boswell (*Agron. J.*, 1968, **60,** 488–493).—Application of Mn (2·23 or 4·46 kg) and B (0·45 or 0·89 kg/ha) to 13 soils increased early and total yields of cotton without affecting lint strength, length or fineness, even though no deficiency symptoms of Mn or B were observed on check plots. Yield increases were negatively correlated with water-sol. soil Mn and B and positively correlated with pH.

A. H. CORNFIELD.

Sprinkler irrigation to protect apricots from frost. E. W. Hewett and J. E. Hawkins (*N.Z. Jl agric. Res.*, 1968, **11,** 927–938).—A pptn. rate of 0·15 in/h appears necessary for protection of apricots when the air temp. at 4·5 ft above ground level falls to 25°F. Sprinklers may safely be turned on when temp. are 2°F above those recommended for firepot lighting during the bloom and post-bloom period. (15 references.) E. G. BRICKELL.

Effect of treatment with adenine and uracil on ribose and deoxyribose content of vine leaves. N. Jákó (*Mitt. Klosterneuburg Rebe u. Wein Obstb. u. Früchteverwert.*, 1968, **18,** 411–415).—Treatment of undefoliated vines with a 50-ppm solution of adenine sulphate increased the ribose : deoxyribose ratio in the leaves within a few weeks. The effect of a 100-ppm solution on defoliated vines was less pronounced. Uracil at 50 ppm also increased the ratio, but decreased it at 100 ppm, irrespective of defoliation. Treatments with both compounds at 50 ppm improved the quality and yields of grapes. (10 references.) P. S. ARUP.

Significance of soil temperature for shoot and flower formation in the vine. J. Blaha (*Mitt. Klosterneuburg Rebe u. Wein Obstb. u. Früchteverwert.*, 1969, **19,** 6–10).—Mean temp., taken over 4 years, were 1·8° above atm. temp. at a soil depth down to 30 cm and 2·3° below atm. temp. at a depth of 40–100 cm. The possible significance of these results is discussed. P. S. ARUP.

Effect of organic manure on chemical and structural characteristics of soil of a banana plantation. J. Godefroy, J.-M. Charpentier and P. Lossois (*Fruits d'outre mer*, 1969, **24,** 21–42).—Treatments with straw (**I**) from forest herbage, with org. manure (**II**) prepared from vegetable refuse of banana culture and grass-litter and with mixtures (**III**) of **I** and **II** were compared. Little difference was found between the effects of **I** and **II** as regards the improved C/N ratio, org. N content and soil structure; the best results were obtained with **III** treatment. The effects were manifest during the 2nd and 3rd year but were not cumulative over 9 years. As the difference between the degrees of soil improvement was not reflected in the banana yields, preference might be given to the (cheapest) treatment with **I**, but **III** might be used on poor soils. (11 references.)

P. S. ARUP.

Varietal response of veneer grafting in mango. S. A. P. Jagirdar, M. H. Nizamani and M. A. Shaikh (*W. Pakistan J. agric. Res.*, 1968, **6,** 86–89).—Comparative trials were made by veneer grafting of 8 varieties to 9-month-old stock. The varieties Sindhri, Langra and Dusehri, as scions, gave the best sprouting results. High R.H. and reduced day temp. benefited the varieties.

P. S. ARUP.

Response of peas to plant population and spacing. E. T. Gritton and J. A. Eastin (*Agron. J.*, 1968, **60,** 482–485).—Over 2 years yields of two varieties of pea increased with increasing plant population ($0{\cdot}55 \times 10^6$ to $1{\cdot}66 \times 10^6$ plants per ha) and with decreasing row spacing (27 cm to 9 cm). A. H. CORNFIELD.

Soil–plant relationships of two steppe desert shrubs in north-west United States. W. H. Rickard and R. F. Keough (*Pl. Soil*, 1968, **29,** 205–212).—When growing in a mixed stand in steppe desert greasewood (*Sarcobatus vermiculatus*) accumulated large amounts of Na, whilst hopsage (*Grayia spinosa*) accumulated large amounts of K. The decomposition of leaf litter beneath the canopy of these shrubs has greatly increased soil exchangeable Na under greasewood and exchangeable K under hopsage compared with adjacent open ground, and has also affected the mineral composition of grass growing in the area. A. H. CORNFIELD.

Blister-like malformations on tea seedlings. C. S. Venkata Ram and K. S. Venkataramani (*Proc. Indian Acad. Sci., B.*, 1968, **68,** 304–307).—It was shown that malformations on tea seedlings in Ceylon and India are in no way connected with the blister blight fungal disease of tea, which is not seed-borne. E. G. BRICKELL.

Cation-exchange capacity of roots and yield potential in sugar-cane. K. Chiranjeevi Rao, T. N. Krishnamurthy and J. Thuljaram Rao (*Pl. Soil*, 1967, **27,** 314–318).—Cane yields (tons/acre) of 11 varieties of sugar-cane were highly correlated with the cation-exchange capacity (*CEC*) of sett roots (30 days after planting) and shoot roots (95–135 days after planting). *CEC* values of sett and shoot roots did not differ significantly, nor the values due to stages. It is suggested that *CEC* is useful as a yield index in large scale progeny testing on this crop. A. H. CORNFIELD.

Influence of gibberellic acid on cigarette tobacco. M. Siddiq, M. A. Aziz and M. S. Khan (*W. Pakistan J. agric. Res.*, 1968, **6**, 76–85).—Spraying tobacco seedlings in the field with gibberellic acid at 10 ppm concn. significantly increased the yield of dry and cured leaf and improved the quality of the tobacco. P. S. ARUP.

Breeding of Hevea. P. K. Wycherley (*Plrs' Bull. Rubb. Res. Inst. Malaya*, 1968, No. 99, 159–170).—An abridged version of review covering the effect of replanting, breeding and selection schedules, yield factors and breeding methods, is presented.
E. G. BRICKELL.

Pest Control

Synthesis and antifungal activity of some carbohydrate and amino acid derivatives of dimethyldithiocarbamic acid and of pyridine-2-thiol 1-oxide. C. W. Pluijgers, J. Berg and G. D. Thorn (*Recl Trav. chim. Pays-Bas*, 1969, **88**, 241–253).—Carbohydrate (β-D-glucose, β-D-ribose, β-D-xylose, β-D-glyceraldehyde, *N*-acetyl-β-D-glucosamine, β-D-methylglucuronate, β-D-methylgalacturonate) deriv. of dimethyldithiocarbamate (**I**), the β-D-methylglucuronate-triacetate deriv. of diethyldithiocarbamate, the β-D-glucose deriv. of pyridine-2-thiol 1-oxide, the α-alanine, α-aminobutyric acid and pyruvic acid deriv. of **I**, and DL-thiazolidine-2-thione-4-carboxylic acid (byproduct) were prepared. The activities of these compounds against cucumber scab (*Cladosporium cucumerinum*) and other fungi were assessed; good systemic activity was given by D-, L-, and DL-β-*N*,*N*-(dimethylthiocarbamoylthio)-α-alanine. (14 references.) (In English.) E. J. H. BIRCH.

Agricultural fungicides. IV. Preparation of 2- and 4-iodo-3,5-dinitrobenzoates. L. A. Summers and K. Lehtonen (*Aust. J. Chem.*, 1969, **22**, 497–498).—The method consists of diazotisation of 2-(or 4-)amino-3,5-dinitrobenzoic acid in conc. H_2SO_4 at $< 10°$, followed by reaction of the solution with KI in aq. AcOH at $< 5°$ to form 2-(or 4-)iodo-3,5-dinitrobenzoic acid, which is then converted into the required Me or Et ester by acid-catalysed esterification. Me 2-iodo-3,5-dinitrobenzoate, m.p. 113° (cryst. from MeOH), Et 2-iodo-3,5-dinitrobenzoate, m.p. 117° (cryst. from EtOH), Me 4-iodo-3,5-dinitrobenzoate, m.p. 164°, and Et 4-iodo-3,5-dinitrobenzoate, m.p. 169°, are being submitted to biological tests, the I atom being very reactive. W. J. BAKER.

Benzo[*b*]thienyl carbamate insecticides. J. R. Kilsheimer, H. A. Kaufman, H. M. Foster, P. R. Drlscoll, L. A. Glick and R. P. Napier (*J. agric. Fd Chem.*, 1969, **17**, 91–93).—The synthesis of 17 such carbamates is described, and their physical and biological properties are tabulated. Benzo[*b*]thien-4-yl methylcarbamate (Mobam) showed a particularly broad spectrum of insecticidal activity with low mammalian toxicity. (26 references.)
P. S. ARUP.

Insecticidal 2-(methylcarbamoyloxyphenyl)-1,3-dioxolanes, -oxathiolanes and -dithiolanes. J. A. Durden, jun. and M. H. J. Weiden (*J. agric. Fd Chem.*, 1969, **17**, 94–100).—Among these compounds, 2-(2-methylcarbamoyloxyphenyl)-1,3-dithiolane, -dioxolane and -oxathiolane were active against aphids, beetles and houseflies; the analogues and homologues were much less active as insecticides although their capacities for acetylcholinesterase inhibition were not greatly reduced. (20 references.)
P. S. ARUP.

Physiologically active compounds. XV. Synthesis of 3-alkylflavones by a modified Baker–Venkataraman transformation. G. Srimannarayana and N. V. Subba Rao (*Indian J. Chem.*, 1968, **6**, 696–699).—Twelve 3-alkylflavones with the isorotenoid skeleton were synthesised to compare their toxicities (to fish) with flavones without the alkyl substituent. In a modified Baker–Venkataraman method, ω-alkyl-resacetophenones and -phloracetophenones were refluxed with aroyl chlorides in acetone with K_2CO_3 as catalyst to give 3-alkylflavones directly. The Me and allyl ethers of hydroxyflavones were prepared. Resacetophenone gave only diketones while phloracetophenone gave 3-aroylflavones. I.r. and u.v. spectra are tabulated. 7,3′,4′-Trimethoxy-3-methylflavone was highly toxic to fish (*Barbo ticto*) and half to a third as toxic as rotenone, and may be useful as an insecticide. (23 references.)
G. W. FLINN.

Movement of pesticides in soil. C. I. Harris (*J. agric. Fd Chem.*, 1969, **17**, 80–82).—The mobilities of 11 insecticides in standard sub-irrigated soil columns were, for chlorinated insecticides nil, for phorate and disulfoton very slow, and for diazinon and thionazin moderately fast, thionazin being the most rapidly moving. The results are compared with earlier results by Harris (*Weeds*, 1967, **15**, 214). P. S. ARUP.

Soya: Herbicides in post-emergence. G. S. Rosas (*Infciones Grasas aceit.*, 1968, **6**, 155–167).—The effect of various herbicides in soyabean cultivation on plant height, yield and seed wt. were examined. When there was lack of moisture in the soil, Amiban (2,4-DB) had a negative effect on all properties, but when moisture was not lacking, Amiban, Debexone and Lorox could be used up to certain concn. without reducing the yield of seed.
L. A. O'NEILL.

Virus diseases of grasses. A. J. H. Carr (*Span*, 1968, **11**, 92–95).—Viruses and hosts, transmission, symptoms and diagnosis, prevalence and effect on crops and control are discussed. Plant breeding appears to offer the only economic prospect of amelioration in the grass crop. E. G. BRICKELL.

Verticillium wilt of tobacco. VI. Influence of roots and stems on Verticillium wilt symptoms. VII. Pathogenicity of isolates of *Verticillium dahliae* Kleb. on New Zealand tobacco. VIII. Movement of conidia of *Verticillium dahliae* Kleb in tobacco plants. D. S. C. Wright, L. N. Gibbins (VI) and J. M. Biss (VII) (*N.Z. Jl agric. Res.*, 1968, **11**, 789–796, 797–802, 803–811).—VI. Data from three cultivars grafted so that the stems and roots were united in all possible combinations showed that 74% of the resistance to *V. dahliae* Kleb. in all varieties at all stages of growth was contributed by the root system, the remainder coming from the stem. (11 references.)

VII. Isolates from one tobacco cultivar from four different fields were compared for virulence in inoculated field trials. No significant differences were found between strains. In the field the growth of infected tobacco was retarded, final leaf no. increased, and internode length shortened. (14 references.)

VIII. Conidia introduced into the roots by inoculation were translocated throughout the roots, stem, and midribs within 1 h, and were lodged progressively more towards the top of the taproot and the base of the stem than the apex. Conidial movement was more limited in field-tolerant cultivars than in the very susceptible or highly resistant ones. (10 references.) E. G. BRICKELL.

Resistance to *Verticillium*: the biochemical approach. M. E. U. Taylor (*Span*, 1968, **11**, 96–99).—*Verticillium* wilt was studied mainly in tobacco. The results showed that fungal spores are carried in the transpiration stream to parts of the plant remote from the site of infection, and there are both short-lived and protracted mechanisms existing in varying degrees in both susceptible and resistant varieties. Rootstock is of prime importance, but the enzymic oxidation of phenols secreted by tobacco roots is also important in resistance to *Verticillium*. (15 references.)
E. C. BRICKELL.

Influence of soil conditions on development of *Rhizoctonia solani* Kühn on tobacco. W. F. T. Hartill (*Rhod. J. agric. Res.*, 1968, **6**, 77–79).—Sore shin disease from the fungal inoculations tended to develop more readily with decreasing soil moisture in glasshouse trials. The presence of root-rot nematodes had no effect on the fungal development. P. S. ARUP.

Metabolism of Chloroneb by *Rhizoctonia solani* and other fungi. W. K. Hock and H. D. Sisler (*J. agric. Fd Chem.*, 1969, **17**, 123–128).—Chloroneb (1,4-dichloro-2,5-dimethoxybenzene) in a nutrient medium for the fungus was slowly converted into a non-toxic compound which was identified by i.r., n.m.r. and mass spectrometry as 2,5-dichloro-4-methoxyphenol. Mycelial growth was stopped for the first 24 h, during which 50% of the toxic insecticide had been converted. Chloroneb was not metabolised by *Sclerotium rolfsii* or *Saccharomyces pastorianus*, but was metabolised to an unknown compound by *Neurospora crassa*. (18 references.)
P. S. ARUP.

Comparative fungitoxicity and phytotoxicity of sodium *N*-methyldithiocarbamate and its n-alkyl homologues. L. T. Richardson and G. D. Thorn (*Can. J. Bot.*, 1969, **47**, 241–245).—Et, Pr and Bu homologues were comparable to the title compound (**I**) in toxicity to *Monilinia fructicola*; the amyl and hexyl deriv. were less active. A further increase in the C chain up to decyl increased fungitoxicity correspondingly. Germination of barley seeds was inhibited progressively less from Me to heptyl, slightly more by octyl, and less by decyl deriv. **I** and the Et homologue were the most effective in controlling pre-emergence damping-off of peas; Pr through octyl were progressively less effective, and nonyl and decyl homologues were completely ineffective. E. G. BRICKELL.

Chemical control of a fruit tree false spider mite, *Cenopalpus pulcher*, in the United Arab Republic: I. Toxicity of three acaricides to egg and adult stages. S. M. Hassan, M. R. Abo-Elghar, E. A.

Elbadry and G. I. Zohdy (*J. econ. Ent.*, 1968, **61**, 1482–1485).—In laboratory tests, tetradifon (0·2%) gave higher kills than dicofol or Thiocron [*O,O*-dimethyl phosphorodithioate *S*-ester with 2-mercapto-*N*-(2-methoxyethyl)acetamide] when sprayed on newly laid eggs of *C. pulcher*. When adult mites were exposed to sprayed leaves, dicofol (0·01%) gave 97% mortality, Thiocron had limited toxicity and tetradifon was almost non-toxic. (13 references.)
C. M. HARDWICK.

Laboratory testing programme for assessing field performance of acaricides against *Tetranychus urticae* (Koch). A. G. Smith (*N.Z. Jl agric. Res.*, 1968, **11**, 863–873).—Tests using demeton-S-methyl (**I**), carbophenothion (**II**) and dinobuton against a suspected organophosphorus-resistant strain and a susceptible strain of *T. urticae* mites are described. The resistant strain had a high level of resistance and a strong avoidance reaction to **I** and a moderate resistance to **II** in comparison with the susceptible strain. An apparatus for dosing mites and equally small first instar larvae with drops of commercially formulated pesticides is also described.
E. G. BRICKELL.

Nitrogen and phosphorus content of greenbugs reared on fertilised wheat. N. E. Daniels, G. C. Wilson and C. S. Clarke (*J. econ. Ent.*, 1968, **61**, 1746–1747).—*Schizaphis graminum* fed on high N- or P-containing wheat absorbed only the amounts of N and P they needed and so contained quantities similar to those fed on wheat with lower levels of nutrients. C. M. HARDWICK.

***In vivo* fate of the insecticide Zectran in spruce budworm, tobacco budworm and housefly larvae.** R. B. Roberts, R. P. Miskus, C. K. Duckles and T. T. Sakai (*J. agric. Fd Chem.*, 1969, **17**, 107–111).—Zectran was highly toxic to the spruce budworm larvae, but not so toxic to the other two larval insects. After treatment of the larvae with Zectran, nine or ten metabolites were found in each type of insect; of these, four were identified as being common to all three larvae. Hydrolysis of Zectran was not observed. The rates of penetration through the insect cuticles were similar for all the insects for the first 3 h, after which the rate for the spruce budworm became much faster than that for the other two insects. (21 references.) P. S. ARUP.

Metabolic fate of Abate insecticide in the rat. R. C. Blinn (*J. agric. Fd Chem.*, 1969, **17**, 118–122).—Most of the radioactivity of a tritium-labelled oral dose of Abate was eliminated in the faeces (chiefly) and the urine, as unchanged Abate. Traces of Abate were found in the urine with small amounts of metabolic products, viz., sulphate ester conjugates of 4,4′-thio-, sulphinyl- and sulphonyl-diphenol. P. S. ARUP.

Metabolism of UC-21149 [2-methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyl)oxime] in cotton plants and soil in the field. D. L. Bull (*J. econ. Ent.*, 1968, **61**, 1598–1602).—Individual leaves of cotton plants were given petiole injections of UC-21149. Conversion to the sulfoxide started almost immediately: the metabolites are listed. When applied to the soil 86% of the radioactivity was lost after 8 weeks. If the solution was applied topically near the base of the plant, 23% of radioactivity was recovered from the fruit and foliage after 2 weeks. Two weeks after injection at the base of mature leaves, 80% of radioactivity was recovered; of this, 93% remained in the treated leaves and the rest was distributed uniformly through the plant. C. M. HARDWICK.

Antifeeding effect of DDT on bollworm and tobacco budworm larvae. H. R. Bullock and D. A. Wolfenbarger (*J. econ. Ent.*, 1968, **61**, 1760–1761).—The effect of feeding toxaphene and DDT in a mixture or individually was evaluated on treated leaf discs with larvae of *Heliothis* spp.; % consumption was lowest on discs treated with DDT and DDT + toxaphene. Many discs treated with DDT were only partially consumed, showing that DDT exhibits an antifeeding reaction. C. M. HARDWICK.

Comparative study of the toxicities of aldrin and dieldrin. A. S. K. Ghouri and R. M. Tasneem (*W. Pakistan J. agric. Res.*, 1968, **6**, 127–140).—As contact poisons against *Gryllodes sigillatus*, the action of dieldrin (**I**) was significantly faster than that of aldrin (**II**), and both were effective in dosages of 0·2%, giving mortalities of 100 and 96%, respectively, after 30 h. As stomach poisons both were equally effective against *Acheta domesticus*, presumably owing to the conversion of **II** into **I** in the digestive system. This oxidative conversion did not occur on filter paper, on exposure to air. P. S. ARUP.

Methyl parathion resistance in a laboratory strain of the bollworm. F. L. Carter and J. R. Phillips (*J. econ. Ent.*, 1968, **61**, 1716–1718).—*Heliothis zea* was selected for methyl parathion resistance over 11 generations. Log dose–probit regression lines are given for each selection and their significance is discussed. LD_{50} values for the final population indicated development of an approx. 8–10-fold level of resistance. C. M. HARDWICK.

Methyl parathion adsorbed on the skin, and blood cholinesterase levels of persons checking cotton treated with ultra-low volume sprays. S. J. Nemec, P. L. Adkisson and H. W. Dorough (*J. econ. Ent.*, 1968, **61**, 1740–1743).—Men taking insect infestation counts within 2 h of spraying of cotton, absorbed substantial amounts of methyl parathion; 24 h after application the amounts absorbed were greatly reduced. Cholinesterase levels in the men were monitored and a marked depression was shown over the whole period. C. M. HARDWICK.

Bioassay evaluation of heptachlor, isobenzan and diazinon used against the alfalfa weevil in northern Utah. D. W. Davis and Lan Lin Wu (*J. econ. Ent.*, 1968, **61**, 1751–1752).—Bioassay of field-collected *Hypera postica* showed weevils with varying degrees of resistance to heptachlor. There was cross resistance among larvae between isobenzan and heptachlor but adults were easily killed by the former. A high tolerance to diazinon, which had only been used once, was found. C. M. HARDWICK.

Molecular structure of nonionic surfactants in relation to laboratory activity. K. E. Maxwell and W. D. Piper (*J. econ. Ent.*, 1968, **61**, 1633–1636).—Fifty nonionic ethers and esters were evaluated as insecticides by determination of the concn. necessary to prevent emergence of adult *Culex pipiens quinquefasciatus*. Alkyl phenol-ethyleneoxide ether (**I**) gave LC_{50} values of 1–2 ppm; alkylaryl sulphonates and alcohol sulphates were less effective than the more active nonionic **I**. Amphoteric surfactants were more active than anionic compounds. (14 references.) C. M. HARDWICK.

Laboratory evaluation of insecticides against white-fringed weevil [*Graphognathus leucoloma*) (Boheman) (Coleoptera:Curculionidae)] larvae. D. H. Todd (*N.Z. Jl agric. Res.*, 1968, **11**, 903–910).—Of 38 insecticides, only dieldrin, dyfonate, heptachlor, lindane, Sandoz 6538 and thionazin were significantly toxic in soil against the larvae. These larvae probably cannot be controlled in established pasture using currently available insecticides.
E. G. BRICKELL.

Subterranean-termites their prevention and control in buildings. R. A. St. George, H. R. Johnston and R. J. Kowal (*Home Gdn Bull., U.S. Dep. Agric.*, 1969, No. 64, 30 pp.).—Appearance, biology and habits of the genus *Reticulitermes* are described, together with methods of preventing their attack during the construction of buildings, their control in buildings by sanitation, structural and chemical methods, and notes on other insects that damage wood. E. G. BRICKELL.

Spider mites on cotton. Anon. (*Leafl. U.S. Dep. Agric.*, 1968, No. 502, 8 pp.).—Control by miticides is discussed.
E. G. BRICKELL.

Controlling potato insects. W. A. Shands, B. L. Landis and W. J. Reid, jun. (*Fmrs' Bull., U.S. Dep. Agric.*, 1969, No. 2168, 16 pp.).—Tabular data are presented on recommended insecticides together with information on uses, care of equipment, mixing, application, precautions and some non-chemical controls. E. G. BRICKELL.

Grasshopper control. Anon. (*Fmrs' Bull., U.S. Dep. Agric.*, 1968, No. 2193, 11 pp.).—Selection, application and dosage rates for insecticides are given together with information on cultural practices and other measures. E. G. BRICKELL.

Wound dressings for the prevention of silver-leaf in fruit trees caused by *Stereum purpureum* (Pers). Fr. M. H. Dye and P. J. Wheeler (*N.Z. Jl agric. Res.*, 1968, **11**, 874–882).—In laboratory and glasshouse tests, wound dressings based on polyvinyl acetate with or without added fungicide performed better than bitumen emulsion with added fungicide (captafol or thiram), and they were easier to apply consistently under field conditions. No phytotoxicity to dormant trees was observed. E. G. BRICKELL.

Pesticides—Special issue of Chem. Age India. (*Chem. Age India*, 1968, **19**).—39 pages including the following: **Pyrethrum – nature's insecticide.** S. Prasad (751–753). **Neem [extract of fruits of the Indian tree *Azadirachta indica*, *Melia azadirachta*] as an insect deterrent.** S. Pradhan and M. G. Jotwani (756–760). **Aureofungin and streptocycline – Hindustan Antibiotics Ltd. products to control plant diseases.** Anon. (761–762). **Rôle of rodenticides in India.** P. J. Deoras (763–769). **Weedicides in India today.** H. G. Singh (770–774). **Herbicides in the modern chemical age.** V. S. Mani (776–779). **Rôle of plant protection in agricultural development.** N. R. Srinivasan (780–782). **Heating phenomenon in grain.** K. V. Hulkopkar (782–783). **Pesticides in food processing industries.**

S. K. Majumder and H. A. B. Parpia (784–788).—(40 references.) **Infestation control in stored products.** S. V. Pingale (794–796). **Seed coatings with pesticides.** B. K. Desai (799–800). **Effect of formulations on the toxicity of insecticides.** M. G. Jotwani (802–807).—(12 references.) **Complete plant for production of DDT and HCH.** H. J. Weidner, K. H. Berger and H. Heinze (808–810). **Recent improvements in the technology of DDT manufacture.** I. S. Rao (811–813). **Manufacture of benzene hexachloride in India.** K. S. Chari, N. M. Singh and R. K. Tawney (814–818).—(14 references.) **Standardisation in the field of pest control.** E. N. Sundar (826–828). **Packaging of pesticides.** S. S. Malhotra (829–832). **Machinery for application of pesticides.** L. M. Patel (840–842). **Advances in spraying techniques.** Shaw Wallace & Co. (842–843). **Malathion *ULV* [ultra-low volume] ground application.** Cyanamid India Ltd. (844–845). **Aerial spraying of pesticides in India.** Anon. (846–847). K. GRAUPNER.

Emergence of parasites associated with the cabbage aphid during a chemical-control programme. G. L. Godfrey and R. B. Root (*J. econ. Ent.*, 1968, **61**, 1762–1763).—Aphid mummies containing larval and pupal stages of aphid parasites, chiefly *Charips brassicae* and *Diaeretiella rapae*, were not usually greatly affected by sprays of carbaryl or cryolite. C. M. HARDWICK.

Field testing candidate insecticides on radish, cabbage, and cauliflower for control of the cabbage maggot in New York State. F. D. Judge, H. B. Rinick, jun. and F. L. McEwen (*J. econ. Ent.*, 1968, **61**, 1572–1577).—Insecticides were tested as broadcast preplanting soil treatment, as in-furrow treatment, and as drenches for control of *Hylemya brassicae*. (21 references.) C. M. HARDWICK.

Population densities of the European red mite and the predaceous mite *Typhlodromus* (A) *fallacis* on apple foliage following treatment with various insecticides. F. C. Swift (*J. econ. Ent.*, 1968, **61**, 1489–1491).—One application of each of 9 insecticides was sprayed on apple trees and the effect measured over 5 weeks. In general there was an inverse relationship between the toxicity of the insecticide to the predator and changes in *Panocychus ulmi* population. The toxicity to *T. fallacis* controlled the numbers of *P. ulmi* rather than the direct effect of the insecticide, and the data show that a single application of certain insecticides can create a serious problem when used regardless of the effect on a beneficial species. C. M. HARDWICK.

Responses of the Pacific spider mite and citrus red mite to laboratory and field applications of tricyclohexyl tin hydroxide. L. R. Jeppson, M. J. Jesser and J. O. Complin (*J. econ. Ent.*, 1968, **61**, 1502–1505).—TCTH was found to be toxic to *Panonychus citri* and *Tetranychus pacificus* (**I**), nymphs being slightly more susceptible than adult females. Toxicity from direct sprays of TCTH was 10× greater than that from its residues. Resistance in **I** was not found in the laboratory through 10 generations. Residues from field-spray applications to lemons remained toxic for 30–60 days, and residues on sprayed fruit persisted longer than on leaves. Washing with detergent removed much of the toxicity showing that it was mostly from surface residues. Severe leaf drop occurred when TCTH was applied immediately after application of 1·2% petroleum oil. C. M. HARDWICK.

Field tests of acaricides against Pacific spider mite on grapevines. E. M. Stafford (*J. econ. Ent.*, 1968, **61**, 1641–1645).—Field tests showed that plots having the highest pre-treatment counts of *Tetranychus pacificus*, also had the highest post-treatment counts. Of 12 insecticides tested, five including Tranid [*exo*-5-chloro-6-oxo-*endo*-2-norbornanecarbonitrile *O*-(methylcarbamoyl)oxime] and Dessin (2-s-butyl-4,6-dinitrophenyl isopropylcarbamate) were promising. C. M. HARDWICK.

Two-spotted spider mite and hop aphid control on cluster hops with acaricides. W. W. Cone (*J. econ. Ent.*, 1968, **61**, 1685–1689).—Of 13 compounds, tested over 2 seasons for control of *Tetranychus urticae* and *Phorodon humuli*, Azodrin (3-hydroxy-*N*-methyl-*cis*-crotonamide dimethyl phosphate and Tranid [*exo*-5-chloro-6-oxo-*endo*-2-norbornanecarbonitrile *O*-(methylcarbamoyl)oxime] gave season long control and were suitable to replace present combination treatments. C. M. HARDWICK.

Toxicity of pesticides to corn earworm on sweet-corn in southern California, 1962–1967). L. D. Anderson and H. Nakakihara (*J. econ. Ent.*, 1968, **61**, 1477–1482).—Many small scale and 6 large scale tests to control *Heliothis zea* were carried out over 6 seasons; results of treatment with 32 materials are tabulated. Gardona [2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate] and carbaryl were equal to or better than DDT for corn earworm control; correct timing of pesticide application was necessary to avoid damage to bees. Earworm resistance to DDT appears to have increased 30- to 50-fold since 1954. C. M. HARDWICK.

Insecticides for control of insects attacking alfalfa [lucerne] seed in eastern Nebraska. S. D. Kindler, G. R. Manglitz and J. M. Schalk (*J. econ. Ent.*, 1968, **61**, 1636–1639).—Azodrin, dimethoate and GC-6506 [dimethyl *p*-(methylthio)phenyl phosphate] were equal or superior to DDT against mirids, leafhoppers and grasshoppers. No treatment was effective against the lucerne seed chalcid (*Bruchophagus roddi*). No constant significant increase in seed yield was obtained with any treatment. C. M. HARDWICK.

Systemic activity of 2-methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyl)oxime (UC-21149) in the cotton plant [against] the boll weevil. R. L. Ridgway, S. L. Jones, J. R. Coppedge and D. A. Lindquist (*J. econ. Ent.*, 1968, **61**, 1705–1712).—Bioassay showed that side dressings of UC-21149 produced the greatest accumulations in older leaves and the least in debracted squares (floral buds). 4 lb/acre (preferably as 2 applications), controlled *Anthonomus grandis* in field cages. Multiple applications resulted in the greatest uptake in new plant growth. Deep placement and irrigation also increased uptake. After soil application, the sulphoxide was the chief metabolite in the squares. (23 references.) C. M. HARDWICK.

Field tests with in-furrow and seed treatments of systemic insecticides on cotton at Stoneville, Mississippi. T. R. Pfrimmer (*J. econ. Ent.*, 1968, **61**, 1607–1612).—Eight systemic insecticides were applied in-furrow and populations of *Frankliniella fusca* were sampled. Granular disulfoton, phorate and three proprietary formulations gave good initial control and UC-21149, [(2-methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyl)oxime] gave best results for residual control. Seed treatment with e.g., disulfoton or methomyl also gave low initial aphid counts. The treatments had no significant effect on beneficial insects or spiders. Thrips control resulted in earlier fruiting in some tests. C. M. HARDWICK.

Residual activity of chlorinated hydrocarbon insecticides in permanent turf for European chafer control. F. L. Gambrell, H. Tashiro and G. L. Mack (*J. econ. Ent.*, 1968, **61**, 1508–1511).—Six compounds were applied as sprays to turf to control *Amphimallon majalis* grubs. Lindane gave > 90% control for 1–4 seasons, Chlordane, heptachlor and aldrin gave > 89% control for 8 seasons and dieldrin completely eliminated the grubs for the same period. C. M. HARDWICK.

Evaluation of topically applied dimethoate for control of lilac leaf miner. N. W. Wilkinson (*J. econ. Ent.*, 1968, **61**, 1746).—Dimethoate applied to the stems of lilac bushes, substantially reduced damage by *Gracillaria syringella*, without phytotoxic effects. Application before and at the time of the first adult flight provided protection all summer; damage was further reduced by a second application later. C. M. HARDWICK.

Control of pinyon needle scale with dimethoate. D. A. Pierce, W. F. McCambridge and G. E. Moore (*J. econ. Ent.*, 1968, **61**, 1697–1698).—Dimethoate (0·5%) spray gave satisfactory control of *Matsucoccus acalyptus* if applied 7–10 days after the red eye spots become visible in the eggs. The addition of the surfactant Triton B 1956 did not improve control. C. M. HARDWICK.

European pine sawfly control with aircraft application of concentrate insecticidal sprays. W. E. Wallner (*J. econ. Ent.*, 1968, **61**, 1666–1667).—Concentrate sprays of oxydemeton-methyl, Dursban (*O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate), Baygon (*o*-isopropoxyphenyl methylcarbamate) and ultra-low vol. sprays of malathion gave good control of *Neodiprion sertifer*. C. M. HARDWICK.

Field tests of systemic insecticides for control of Sitka-spruce weevil. N. E. Johnson and J. G. Zingg (*J. econ. Ent.*, 1968, **61**, 1650–1652).—Bidrin (3-hydroxy-*N,N*-dimethyl-*cis*-crotonamide dimethyl phosphate) (**I**) and oxydemeton-methyl (**II**) sprayed on to leading shoots of *Picea sitchensis* in spring gave good control of *Pissodes sitchensis*. When tested by injection schradan and demeton were not effective but **I** and **II** were promising. C. M. HARDWICK.

White-pine weevil control in plantations with heptachlor granules. J. O. Nichols (*J. econ. Ent.*, 1968, **61**, 1543–1546).—Heptachlor granules were applied in a 2-ft radius of trunks of *Pinus strobus*; 5% granules gave as good a control of *Pissodes strobi* as 10% and treatment was effective for at least 5 years. Max. effectiveness was 95%, in the second year. C. M. HARDWICK.

Relative effectiveness of Tropital and piperonyl butoxide as synergists for pyrethrins against stored-product insects. L. L.

McDonald (*J. econ. Ent.*, 1968, **61**, 1645–1646).—A 1 : 10, pyrethrins : synergist ratio was more effective against *Tribolium confusum* adults than were lower ratios. Pyrethrin + piperonyl butoxide was more effective than pyrethrin + Tropital (piperonyl bis[2-(2-butoxyethoxy)ethyl]acetal) at the LD_{50} level against *Tribolium confusum* and *Lasioderma serricorne* adults and larvae of *Attagenus megatoma*. At the LD_{50} level pyrethrin + Tropital was more effective only against *T. confusum* adults. C. M. HARDWICK.

Thermotherapy. Method for direct cure of virus-infected fruit trees. C. Blattný (*Mitt. Klosterneuburg Rebe u. Wein Obstb. u Früchteverwert.*, 1968, **18**, 455–461).—Various heat therapy treatments are reviewed. Successful results were obtained in treatments of *Juglans regia* seedlings at 38° for 2–3 weeks in a box containing ~ 100 plants, surrounded with wood shavings. (25 references.) P. S. ARUP.

Application of pesticides to tobacco seed-beds. W. F. T. Hartill (*Rhod. J. agric. Res.*, 1968, **6**, 71–76).—The effects of various types of nozzles were compared for the spraying of tobacco seedlings. Nozzles with high flow rates tended to cause excessive run-off and soaking, and small nozzles caused damage at high flow rates and when held low over the plants. The safest course was to use medium-sized nozzles at moderate pressures or held well over the plants so that most of the spray adhered to the aerial parts. P. S. ARUP.

A method of evaluating pesticide-application equipment for Florida citrus. R. C. Bullock, R. F. Brooks and J. D. Whitney (*J. econ. Ent.*, 1968, **61**, 1511–1514).—Citrus trees were sprayed with dilute, concentrate, or aerial sprays containing a green luminescent compound. Leaf samples (50) were collected randomly and the spray coverage was assessed in u.v. light. The method is adaptable to other crops but spray ratings must be reassessed. C. M. HARDWICK.

Trapping *Hylemya* spp. flies in tobacco fields on sticky coloured stakes. J. B. Kring (*J. econ. Ent.*, 1968, **61**, 1567–1569).—Stakes painted in different colours and covered with Stickem, were set up in a field. White and yellow stakes captured the most flies, black the least. Placing fertiliser in piles round the base of the stakes did not increase the total number of flies captured but did collect them. Attraction of the flies to yellow stakes is thought to be a feeding site response. (17 references.) C. M. HARDWICK.

Control of ground-nesting yellow jackets with toxic baits: a five-year testing programme. C. D. Grant, C. J. Rogers and T. H. Lauret (*J. econ. Ent.*, 1968, **61**, 1653–1656).—Three different types of bait dispenser are described. A horsemeat bait containing 1% chlordane gave highly effective control of *Vespula* spp. (1 dispenser per 2 acres) in park and suburban areas. C. M. HARDWICK.

Chemosterilant effects of tepa, apholate and metepa on the alfalfa weevil. J. R. McLaughlin and R. G. Simpson (*J. econ. Ent.*, 1968, **61**, 1730–1733).—Field-collected male *Hypera postica* were immersed in 0·5–2% tepa, apholate or metapa solutions. Using hatch inhibition as an indication of sterility, tepa was the most effective. Male sterility was at max. 9–12 days after immersion. Immersion of females in tepa and apholate stopped egg production. Immersion did not produce toxic effects, but longevity of treated males was significantly reduced. (12 references.) C. M. HARDWICK.

Chemosterilisation of tobacco budworm: a survey of sixteen compounds fed to adult moths. H. M. Flint, W. Klassen, J. F. Norland and E. L. Kressin (*J. econ. Ent.*, 1968, **61**, 1726–1729).—Aziridines, phosphoramides, sulphonates and a nitrogen mustard were administered orally to male and female *Heliothis virescens*; mating, egg hatch, longevity and fecundity were recorded. Only some of the aziridines sterilised both sexes; tretamine being the most effective. Females required bigger doses than males. Phosphoramides were ineffective. (20 references.) C. M. HARDWICK.

Reproduction, longevity, and flight of cabbage looper moths treated topically with tepa. T. J. Henneberry, A. N. Kishaba, M. Z. Iqbal and B. B. Klingler (*J. econ. Ent.*, 1968, **61**, 1536–1540).—Tests were carried out to determine the dosage necessary to produce complete sterility when applied at the junction of the thorax and first abdominal segment, or to the tarsi. Females were twice as tolerant as males. Flight characteristics were not affected by tepa but forced flight after treatment reduced sterility. C. M. HARDWICK.

Apholate-induced sterility in *Bracon hebetor*. L. R. Valcovic and D. S. Grosch (*J. econ. Ent.*, 1968, **61**, 1514–1517).—Topical application and injection of 0·1 or 0·01% apholate solution reduced the fecundity of adult *B. hebetor* but surface contact was much less effective. The relationship between dosage and hatchability was not evident in topical or surface contact treatments. Hatchability showed the same correlation with method of treatment as did fecundity. (11 references.) C. M. HARDWICK.

Histological studies of ovaries in rats treated with [the insect sterilants] hydroxyurea, triphenyltin acetate, and triphenyltin chloride. D. W. Newton and R. L. Hays (*J. econ. Ent.*, 1968, **61**, 1668–1669).—The compounds were given orally for up to 24 days. Hydroxyurea produced no effect on the ovaries, or other side-effects. Triphenyltin compounds decreased the number of mature follicles and decreased ovulation and consequently fertility. C. M. HARDWICK.

Attractants for the Japanese beetle. W. E. Fleming (*Tech. Bull. U.S. Dep. Agric. Res. Serv.*, 1969, No. 1399, 87 pp.).—Of the odoriferous constituents of plants attacked by *Popillia japonica* Newman, and of other chemicals, the most attractive essential oils appeared to be citronella, clove, lemongrass, palmarosa, sassafras, and tansy and the most attractive chemicals were citral, citronellal, eugenol, eugenol methyl ether, geraniol and geranyl acetate. The most attractive mixtures to the beetle contained eugenol mixed with various combinations of anethole, caproic acid, geraniol, β-phenylethyl-acetate, -alcohol and -butyrate, phenyl isovalerate, and isovaleric acid. Fermented baits such as apple or orange juice, or malt or cane sugar syrup, although not desirable for general use, were more attractive to the beetle than the unfermented materials, but extracts of female beetles or traps baited with virgin and field-collected females did not attract either sex. A mechanical trap consisting of a funnel, a four-winged baffle mounted above and extending into the funnel, a bottle-and-wick dispenser for the attracting bait mounted in the bottle, and a receptacle for for holding captured beetles is described; it was of great value in determining the presence or absence of beetles in areas not known to be infested. (106 references.) E. G. BRICKELL.

Behaviour of irradiated boll weevils. I. Feeding, attraction, mating and mortality. II. Reproduction and mortality in cages with untreated boll weevils. A. C. Bartlett and (I only) P. A. Hooker and D. D. Hardee (*J. econ. Ent.*, 1968, **61**, 1677–1680; 1680–1684).—I. For the first 5 days there was no difference between normal and irradiated (6388 or 12,775 rad of ^{60}Co γ-radiation) *Anthonomus grandis*. By 7 days the beetles were becoming inactive and were all dead after 14 days. (16 references.)

II. Both levels of radiation sterilised the weevils but egg hatch was reduced only when the ratio of treated (*T*) to normal (*N*) male weevils exceeded 10 *T* : 1 *N*. Groups of treated females only had no effect on hatch at any ratio. In a 35-day test, four releases of irradiated weevils to maintain a population of 100 *T* : 1 *N* caused a reduction in hatch of 70–90%. C. M. HARDWICK.

Sub-lethal gamma effects on prepupae, pupae and adults of Angoumois grain moth. Z. A. Qureshi, D. A. Wilbur and R. B. Mills (*J. econ. Ent.*, 1968, **61**, 1699–1705).—Young pupae were more susceptible than older ones to irradiation with ^{60}Co. Irradiation of prepupae and 1-day old larvae decreased emergence and caused structural deformities. No dose tested on late pupae significantly affected adult emergence, but irradiation of early pupae at 20 kr can cause complete sterility. When male and female moths, 1 day old, were irradiated, egg deposition and hatchability were reduced; the males remained fertile at all doses. (16 references.) C. M. HARDWICK.

Irradiation of Queensland fruit fly pupae to meet quarantine requirements. E. Shipp and A. W. Osborn (*J. econ. Ent.*, 1968, **61**, 1721–1729).—Pupae of 7 strains of *Dacus tryoni* were irradiated; pupae became most resistant to radiation for the last $\frac{3}{10}$ of their development. Dosages of 5 krad nearly sterilised both sexes and treatment with 10 krad allowed only 6 eggs per million to hatch and none to survive when irradiated males were mated with normal females. (16 references.) C. M. HARDWICK.

Biological control of the bollworm and the tobacco budworm by arthropod predators affected by insecticides. P. D. Lingren, R. L. Ridgway, C. B. Cowan, jun., J. W. Davis and W. C. Watkins (*J. econ. Ent.*, 1968, **61**, 1521–1525).—The effects of foliar application of trichlorfon, Bidrin, phosphamidon and toxaphene + DDT on numbers of *Heliothis* spp., *Psallus seriatus*, and their predators were recorded over 2 seasons. C. M. HARDWICK.

Radioisotopes—a potential means of evaluating the host specificity of phytophagous insects. D. M. Maddox and M. E. Resnik (*J. econ. Ent.*, 1968, **61**, 1499–1502).—*Alternanthera phylloxeroides* absorbed ^{32}P from a solution and translocated it to the leaves within 48 h. Adult male *Agasicles* n. sp. and alligatorweed

thrips (n. sp.) fed on them. From counts of radioactivity, it was shown that allowing for certain corrections, the thrips consumed 2·13 times as much as the flea beetles. C. M. HARDWICK.

Persistence of diazinon and Zinophos in soil: effects of autoclaving, temperature, moisture and acidity. L. W. Getzin (*J. econ. Ent.*, 1968, **61**, 1560–1565).—Diazinon and Zinophos (*O,O*-diethyl *O*-2-pyrazinyl phosphorothioate) were added to a silt loam and their degradation was followed for 16 weeks using g.c. The degradation of diazinon was greatest at higher temp., soil moisture and greater acidity. Zinophos was degraded fastest at pH 7. Autoclaving did not affect the degradation of diazinon but reduced that of Zinophos. (11 references.) C. M. HARDWICK.

Reduction of residues of heptachlor and chlordane in carrots with soil applications of activated carbon. J. F. Ahrens and J. B. Kring (*J. econ. Ent.*, 1968, **61**, 1540–1543).—Both in the glasshouse and in the field, levels of heptachlor and chlordane in carrots were reduced by thorough mixing of the soil with activated C before planting, when the pesticide levels were relatively high. In one field test, where the soil contained long-term low residues, addition of C did not reduce pesticide levels in carrots, possibly due to insufficient mixing. Activated C had no effect on the uptake of DDT by carrots or radishes. Plant growth was not affected. (18 references.) C. M. HARDWICK.

Rapid methylation of chlorophenoxyacetic acid herbicides with dimethyl sulphate for gas chromatographic analyses. J. E. Scoggins and C. H. Fitzgerald (*J. agric. Fd Chem.*, 1969, **17**, 156–157).—A solution of the herbicide in Et_2O, dried with Na_2SO_4 is treated with a 5% solution of Me_2SO_4 in MeOH for 10 min at 55°. After the addition of NaCl the methylated product is extracted with hexane for g.l.c. with γ-hexachlorocyclohexane as internal standard. (11 references.) P. S. ARUP.

Gas chromatographic determination of Abate using flame-photometric and electron-capture detectors. W. E. Dale and J. W. Miles (*J. agric. Fd Chem.*, 1969, **17**, 60–62).—Abate was determined in water (acidified with HCl) by extraction with hexane, and by g.l.c. of the conc. extract with flame-photometric detection. The method was sensitive to 2 mg of Abate as P or 40 mg as S, without any clean-up. Determination by electron-capture detection was less sensitive. Recoveries were 94–103% in the 0·003–1·2 ppm range. (12 references.) P. S. ARUP.

Determination of residues of methyl- and dimethyl-carbamate insecticides by gas chromatography of their 2,4-dinitroaniline derivatives. E. R. Holden, W. M. Jones and M. Beroza (*J. agric. Fd Chem.*, 1969, **17**, 56–59).—The carbamates, extracted from the crop with CH_2Cl_2, and cleaned by a coagulation step, are hydrolysed with KOH, and the liberated amines are treated with 1-fluoro-2,4-dinitrobenzene to form dinitroaniline deriv. for g.l.c. analysis with electron-capture detection. Recoveries of a no. of insecticides from various crops were 90–100%. The background interference was slight. (18 references.) P. S. ARUP.

Thin-layer chromatographic determination of Bidrin, Azodrin and their metabolites. B. Y. Giang and H. Beckman (*J. agric. Fd Chem.*, 1969, **17**, 63–69).—The determination of the two insecticides and nine of their metabolites by modified t.l.c. (in which the plates were scraped to make a no. of narrow parallel straight strips) on silica gel G or H was carried out with 27 solvent systems. Determinations using more than one system were necessary for separating all the compounds. Detection of amounts down to 1 μg was possible by applying 4-(*p*-nitrobenzyl)pyridine to the chromatogram, followed (after drying and heating) by tetramethylenepentamine (both in Me_2CO solution), as chromogenic reagents. R_F values are tabulated for the above compounds and for 38 dyes used in locating the insecticide compounds without the use of chromogenic reagents. (15 references.) P. S. ARUP.

Determination of phorate and five of its metabolites in corn [maize]. M. C. Bowman, M. Beroza and J. A. Harding (*J. agric. Fd Chem.*, 1969, **17**, 138–142).—For the determination of phorate (Thimet) and the metabolites, chopped samples of crops were extracted (Soxhlet) under N_2 for 8 h with $CHCl_3$–MeOH (9 : 1). A solution of the extracts in C_6H_6 was submitted to column chromatography on silica gel and separated into three fractions containing (*i*) phorate and its sulphone, (*ii*) the sulphoxide of phorate and the sulphide and sulphone of the *O*-analogue, and (*iii*) the sulphoxide of the *O*-analogue. The conc. fractions were transferred to C_6H_6 for g.l.c. with a flame-photometric detector sensitive to P. Recoveries were generally > 96%, but only 62% for the *O*-analogue of phorate. The sensitivity was ⩾ 0·004 ppm. Residues were determined in various parts of the plant, at intervals. The relative toxicities of the metabolites to the European corn borer, *Ostrinia nubialis* (Hubner) were determined. (32 references.) P. S. ARUP.

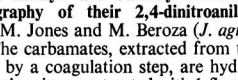
[Pesticidal] esters of thiophosphoric acid. Takeda Chemical Industries Ltd. (B.P. 1,132,097, 7.12.65. Jap. 7.12.64).—Phosphoric esters of formula $(R^1O)_2P(S)\cdot S\cdot CH(CH_2COR^2)COR^3$ are claimed as the active constituents of pesticidal compositions for use in agriculture, gardening, stock-raising and sanitation, where R^1 is lower alkyl and one of R^2 and R^3 is lower alkoxy and the other is a radical of formula $-N(R^4)COOR^5$, where R^4 is H or lower alkyl and R^5 is lower alkyl. In an example *O,O*-diethyl *S*-[1-ethoxycarbonyl-2-(*N*-ethoxycarbonyl-*N*-methylcarbamoyl)-ethyl] phosphorodithioate is prepared from monoethylfumarate mono(*N*-methyl-*N*-ethoxycarbonyl)amide, hydroquinone and *O,O*-diethyldithiophosphoric acid, by heating at 65° for 24 h under anhyd. conditions. S. S. CHISSICK.

Agricultural antimicrobial compositions. Wagaraw Liquidating Corp. (B.P. 1,135,949, 10.12.65. U.S., 10.12.64) Addn. to B.P. 936,342).—Antimicrobial compositions suitable for treatment of soil, seeds, plants, and having a growth stimulating action, contain as active constituent the salicylate salt of a hydroxyquinoline ('oxine') ester of an org. carboxylic acid (e.g., benzoic) in which the nucleus of the salicylic acid radical is devoid of any additional substituent containing acidic H and the oxine is substituted with one or more of the following: halogen, NO_2, 1–8 C-alkyl or -alkoxy. Particularly effective are: di-8-hydroxyquinoline phthalate salicylate, 8-cinnamoyloxyquinoline 3,5-di-iodosalicylate and 5,7-dibromo-8-benzoyloxyquinoline 3,5-di-iodosalicylate. S. S. CHISSICK.

[Pesticidal] substituted benzoxazolone derivatives. CIBA Ltd. (B.P. 1,136,107, 2.2.66. Switz., 3.2.65).—2-Oxo-2,3-dihydrobenzoxazoles substituted in the 3-position by -CY-NRR^1 and optionally in the benzene nucleus by 1–2 halogen, have strong bactericidal and fungicidal properties (effective against, e.g., *Alternaria solani*, *Phytophthora infestans*, *Septoria apii*, *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger*, *Rhizopus nigricans*) (Y is O or S, R and R^1 are H, C_{1-4}-alkyl, or Ph which may contain 1–2 halogen, C_{1-4}-alkyl or -halogenoalkyl). An example is 5-chloro-3-dimethylcarbamyl-2-oxobenzoxazoline, m.p., 131–132°, prepared in 79% yield by boiling a 1 : 1 : 1-mol. mixture of 5-chloro-2-oxobenzoxazoline, NEt_3, and NMe_2COCl in dioxan during 48 h. F. R. BASFORD.

Microbiocidal sulphones. Agripat S.A. (B.P. 1,136,514, 4.7.67. Switz., 5.7.66).—2-Hydroxy-4-halogeno-diphenyl sulphones, useful for protecting plants, fruits, seeds, etc. from attack by fungi and bacteria are claimed. The halogen is F, Cl or Br and the phenyl rings may be further substituted by halogen or lower alkyl groups. In an example, 2-hydroxy-4,4′-dichlorodiphenyl sulphone (**I**) is prepared from the corresponding 2-amino compound (prep. given) by diazotisation at 20–35°, followed by thermal decomposition of the diazo compound at 130–135° and dilution with water. **I** has a m.p. of 125–127°. S. S. CHISSICK.

Nematocidal and fungicidal cyclic imide derivatives. Schering A.-G. (B.P. 1,131,334, 28.6.66. Ger., 11.8.65).—The deriv. have the formula



$CR^IR^{II}\cdot CR^{III}R^{IV}\cdot CO\cdot N\cdot O\cdot CONHR$ wherein R is alkyl, and R^I–R^{IV} are H or Me; or R^I and R^{III} together represent a bond, and R^{II} and R^{IV} are H or Me or form together part of a cycloaliphatic or aromatic ring. An example, is *N*-(methylcarbamyloxy)-phthalimide, m.p. 202–204°, obtained in 95% yield by interaction of *N*-hydroxyphthalimide in tetrahydrofuran with MeNCO in presence of NEt_3 at 30°. F. R. BASFORD.

Substituted oxathiolones. Farbenfabriken Bayer A.-G. (Inventors: E. Mühlbauer and W. Weiss) (B.P. 1,132,109, 18.1.66. Ger., 18.3. and 23.9.65).—The title compounds are fungitoxic agents prepared by interaction of X·COSX (X is halogen) with $R^1\cdot COCH_2R$ (R and R^1 are alkyl, cycloalkyl, aryl, aralkyl, heterocyclyl, or together represent part of a 5- or 6-membered alicyclic or heterocyclic ring, or R is H, halogen, CO_2Et, Ac, or Bz, or R^1 is alkenyl). E.g., a mixture of $COEt_2$ and ClCOSCl is slowly heated to 80°, and when most of the HCl formed has been evolved heating is continued during 1 h to 100°. Distillation then affords 4-methyl-5-ethyl-2-oxo-1,3-oxathiole (75·3%), b.p. 68°/0·9 mm. A solution of this (0·1 g) in potato dextrose agar (1 l.) is 100% inhibitory to the growth of *Cortitium rolfsii*, *Sclerotinia sclerotiorum*, *Thielaviopsis basicola*, *Phytophthora cactorum*, *Fusarium culmorum*, and *F. oxysporum*. F. R. BASFORD.

Carbonic acid esters. Agripat S.A. (B.P. 1,134,314, 30.6.67. Switz., 1.7.66).—The esters, viz., $CO_2R \cdot O[CH_2]_2SCOR^1$, are toxic to phytopathogenic fungi (R is alkyl of 1–4 C, R^1 is Me substituted by 1–2 Cl or Br if desired). In an example, $CH_2Cl \cdot COCl$ is added during 30 min at 10–15° to a solution of $CO_2Et \cdot O[CH_2]_2SH$ in benzene, followed by NEt_3 dropwise. The mixture is stirred at room temp., then the filtered liquor is washed with water, saturated aq. $NaHCO_3$, and more water. Distillation affords Et [2-(chloroacetylthio)ethyl] carbonate, b.p. 104–105·5/0·04 mm. Its activity against *Alternaria tenuis*, *Botrytis cinerea*, *Clasterosporium* sp., *Coniothyrium* sp., *Fusarium culmorum*, *Rhizopus nigricans*, *Penicillium* sp., and *Stemphylium* sp. is described. F. R. BASFORD.

Fungicidal compositions. Agripat S.A., Assee of R. Menassé and K. Gaetzi (B.P. 1,136,776, 13.12.65. Switz., 14.12.64).—Compositions active against e.g., *Alternaria tenuis*, *Botrytis cinerea*, comprise dusts, coated or impregnated granules, wettable powders, solutions, etc., containing as active ingredient a *N*-benzylidene alkylamine of formula $(R^1)_x(R^2)_y \cdot C_6H_{6-n} \cdot (CH:NR^3)_z$ (**I**) in which R^1 is halogen, nitro or hydroxyl or 1–4 C alkyl; R^2 is halogen, nitro, amino, hydroxyl, cyano, thiocyano, alkyl, halogenoalkyl, alkylamino, dialkylamino, alkoxy or alkylthio (alkyls are 1–4 C), carboxyl or carbalkoxy; R^3 is 8–16 C alkyl or alkenyl or 8–18 C alkyl substituted by halogen, hydroxyl or amino, x is 0–3, y is 0–2, z is 1 or 2 and $(x + y + z) = n$ and is $\geqslant 6$. **I** are obtained by reacting, e.g., 2,4-dichlorobenzaldehyde in benzene with tetradecylamine, giving *N*-(2,4-dichlorobenzylidene)-tetradecylamine, m.p. 40°. J. M. JACOBS.

Fungicidal and bactericidal compositions. Agripat S.A. (B.P. 1,136,793, 10.6.66. Switz., 11.6.65).—5-Amino-1,2-dithiol-3-ones (**I**) which are active against e.g., phytopathogenic fungi, have halogen or optionally 1–3 C alkyl-substituted phenyl in the 4-position and the N atom is optionally mono- or di-substituted with 1–14 C alkyl (which may also be substituted with various groups) or the N atom may form part of a 5–7 membered (substituted) heterocyclic ring. Prep. is e.g., from the corresponding 4,5-dichloro-1,2-dithiol-3-one by reaction with an appropriate amine. **I** claimed include 4-chloro-5-pyrrolidino-1,2-dithiol-3-one, 4-chloro-5-morpholino-1,2-dithiol-3-one and 4-chloro-5-hexamethyleneimino-1,2-dithiol-3-one. S. S. CHISSICK.

Herbicidal compositions. Upjohn Co. (Inventor: A. J. Lemin) (B.P. 1,131,022, 23.12.65).—A method of preventing germination of plant seeds and combatting the growth of plants is claimed, which comprises applying a composition containing *N*-alkyl- or *N,N*-dialkyl-3-chlorobenzamide in which the phenyl group is optionally further substituted by an additional 1 or 2 Cl atoms. The alkyl substituents are chosen from Et, Pr^n and Pr^i.
S. S. CHISSICK.

Thiazole derivatives and their use as herbicides. Produits Chimiques Pechiney-St. Gobain (B.P. 1,131,207, 5.4.66. Fr., 6.4.65).—The thiazoles are substituted in the 2-position by $-NR^1 \cdot CZ \cdot NR^{II}R^{III}$ and optionally elsewhere by halogen, SCN, CN, CO_2H, carbalkoxy, NH_2, OH, OH-alkyl, aryl (which may contain halogen, alkyl, and/or alkoxy), aryloxy (optionally containing halogen or alkyl), or C_{1-4}-alkyl or -alkoxy (both of which may contain halogen) (Z is O or S; R^I–R^{III} are H, alkyl, alkenyl, or alkynyl of $\geqslant 4$ C, or R^I is optionally halogenated alkylacyl or -arylacyl, alkylarylacyl, or alkoxyarylacyl, or R^{II}–R^{III} are alkoxy or alkenoxy of 1–4 C, or optionally halogenated alkylaryl, aryl, or aryloxy—but they are not simultaneously H. In an example, MeNCO is added dropwise to a solution of 5-chloro-2-aminothiazole in Me_2SO, temp. rising to 56°. After cooling to 45° and keeping thereat during 1 h, solvent is removed *in vacuo*, the residue is boiled in acetone in presence of C, and the filtered liquor is cooled, to give 1-(5-chlorothiazol-2-yl)-3-methylurea (50%), m.p. 271–271·5°. F. R. BASFORD.

Herbicidal pyridazone derivatives. Badische Anilin- & Soda-Fabrik A.-G. (B.P. 1,131,473, 8.2.66. Ger., 16.2.65).—*N*-[4-(1-phenyl or -cycloalkyl-5-bromopyridazone-6)-yl]-*N'*-methyl or -dimethylformamidines are claimed as the active constituents of selective or total herbicidal compositions, e.g., suitable for controlling unwanted vegetation among crop plants.
S. S. CHISSICK.

Herbicidal isothiazole derivatives. Schering A.-G. (B.P. 1,131,607, 5.5.66. Ger., 8.5.65).—3-Methyl-isothiazoles substituted in the 5-position by a $-NH \cdot C(X)NR^1R^2$ group, where X is O or S, R^1 is H or lower alkyl and R^2 is lower alkyl or cycloalkyl, are claimed as the active constituents of selective herbicidal compositions, e.g., suitable for destroying weeds in the presence of onions, leeks or chives. In an example 3-methyl-5-(*N*-methylcarbamoylamino)-isothiazole (m.p. 217–219°) is prepared from 3-methyl-5-aminoisothiazole by reaction with MeNCO in tetrahydrofuran. S. S. CHISSICK.

[Herbicidal] 3-azabicyclo[3,2,2]nonane derivatives. Monsanto Co. (Inventor: J. J. D'Amico) (B.P. 1,133,975, 6.5.66).—The deriv. contain halogenomethylcarbonyl (halogenoacetyl) in the 3-position, the halogen atom being Cl, Br or I. In an example, $CH_2Cl \cdot COCl$ is added dropwise to a mixture of 3-azabicyclo[3,2,2]-nonane, NEt_3 and ether at 25–30°, then 24 h later water is added. Work-up of the org. layer affords 3-chloroacetyl-3-azabicyclo[3,2,2]nonane (71%), m.p. 74–75° (heptane). F. R. BASFORD.

Herbicidal compositions. Fisons Pest Control Ltd. (Inventor: R. K. Pfeiffer) (B.P. 1,135,563, 30.3. and 30.11.65).—An optionally substituted phenoxyaliphatic acid (or salt, ester, or amide thereof) is compounded (0·1–15) with a $1\text{-}R^I\text{-}2\text{-}X\text{-}4\text{-}R^{II}\text{-}5\text{-}R^{III}\text{-}6\text{-}R^{IV}\text{-}7\text{-}R^V$-benzimidazole (1 pt.), to provide a synergistic herbicidal composition (R^1 is H, alkyl, or CO_2R; R is optionally substituted alkyl or aryl; R^{II}–R^V are H, alkyl, OH, alkoxy, NO_2, halogen, pseudohalogen, SH, alkylthio, substituted alkyl, optionally substituted $CONH_2$ or NH_2, SO_3H, SO_2NH_2, esterified SO_3H, CO_2H, etc., and X is CF_3 or C_2F_5). A representative mixture is 4,5-dichloro-2-(trifluoromethyl)benzimidazole (1) and $4,2,1\text{-}ClC_6H_3Me \cdot O \cdot CH_2\text{-}CO_2H$ (1 pt.). F. R. BASFORD.

Acid anilides and herbicidal compositions containing them. Badische Anilin- & Soda-Fabrik A.-G. (Inventors: G. Steinbrunn and A. Fischer) (B.P. 1,136,633, 2.3.66. Ger., 3.3.65).—Acid anilides (**I**) containing a :N(OH) group and their use for selective weed control, are claimed. **I** have the general formula $X_n \cdot C_6H_{(5-n)} \cdot N(OH)CO \cdot R$, where X is a halogen or alkyl, R is cycloalkyl, n-alkyl or -alkenyl optionally halogen- or Me-substituted and n is 0, 1, 2 or 3. Prep. is from the corresponding hydroxylamine compound by reaction with RCOCl. Thus, 3,4-dichlorophenylhydroxylamine in C_6H_6 is treated with cyclopropane carboxylic chloride in presence of $NaHCO_3$ and under N_2, at 15–20° to give **I** of m.p. 123–125°. S. S. CHISSICK.

2,4,6-Trichloro-4'-nitrodiphenyl ether. Mitsui Kagaku Kogyo K.K. (B.P. 1,136,637, 22.9.66. Japan, 27.9.65).—The title compound, useful as a herbicide, is prepared by purifying 2,4,6-trichlorophenol [obtained by chlorinating phenol, and containing chlorocyclohexadienone (**I**) as impurity] by reducing **I** with an aq. solution of a reducing agent (e.g., a sulphite), reacting the purified phenol with an alkali metal hydroxide (e.g., KOH), and condensing the salt so formed with *p*-nitro-chlorobenzene at 235–240° for 6–7 h.
E. ENOS JONES.

Animal Husbandry

Hydroponic grass. D. Shead (*Dairy Fmr, Ipswich*, 1969, **16**, No. 3, 22–25).—The advantages of producing grass hydroponically as a feed supplement for cows (1–2 lb of grass/100 lb body wt., together with barley straw and roughage) include economy, elimination of the need for compound concentrates, reduced labour time (0·5–1 h per day), and palatability to the cow. P. P. R.

Selenium content of forage and hay crops in the Pacific Northwest (U.S.A.). D. L. Carter, M. J. Brown, W. H. Allaway and E. E. Cary (*Agron. J.*, 1968, **60**, 532–534).—The Se contents are presented in relation to geographical distribution. The ability of the feedstuffs to supply adequate Se to prevent white muscle disease in lambs and calves is discussed. A. H. CORNFIELD.

Performance and vitamin A status of sheep grazing high-nitrate pastures. P. B. O'Donovan and A. Conway (*J. Br. Grassld Soc.*, 1968, **23**, 228–233).—Wt. gains of sheep from March to Aug. were increased significantly by application of Ca NH_4 nitrate (184 lb N per acre) in six split applications to a perennial ryegrass–white clover sward. A second increment of 184 lb N resulted in a further, though non-significant, increase in wt. gains. Herbage NO_3^-% increased with level of N applied and with advancing season. The treatments had no significant effect on liver storage of vitamin A.
A. H. CORNFIELD.

Effects of sheep treading on the herbage and seed yield of four grasses grown in association with white clover. K. R. Brown (*N.Z. Jl agric. Res.*, 1968, **11**, 883–890).—Herbage yields of perennial ryegrass and timothy were not reduced by treading in any season, those of cocksfoot were reduced by winter treading at the highest rate, and yields of browntop were reduced in winter by all rates of treading, and in autumn at the highest rate. White clover yields were reduced by treading in summer at the highest rate and in

winter at all rates. White clover yielded highest when associated with browntop. Yields of perennial ryegrass and timothy were increased by treading, browntop seed yields were reduced and those of cocksfoot not affected. (14 references.) E. G. BRICKELL.

Use of tallow and fats in the rations for broilers. Net, gross and metabolisable energy. Anon. (*Infciones Grasas aceit.*, 1968, **6**, 191–199).—The effect of tallow, animal fat and soyabean oil in a ration (maize, soya flour, fish meal) for broiler fowls on the total and metabolisable energy and net energy for production was studied. Once the animal fats had passed the intestinal wall, they were metabolised with greater facility and less calorific loss than the vegetable oil, carbohydrates and protein. The stability of the animal fats in the ration was greater than that of the lipids in the control ration. L. A. O'NEILL.

The farm beef herd. E. J. Warwick and P. A. Putnam (*Fmrs' Bull., U.S. Dep. Agric.*, 1969, No. 2126, 16 pp.).—Systems of farm beef production, selection of stock, and feeding and care of the herd are described. P. P. R.

Effect of moulded alfalfa [lucerne] hay on rumen activity, performance and digestibility in dairy steers. G. P. Mohanty, N. A. Jorgensen, R. M. Luther and H. H. Voelker (*J. Dairy Sci.*, 1969, **52**, 79–83).—Mould development in lucerne hay was shown to affect its chemical composition and nutrient availability and the rumen protozoa population and performance of animals fed the hay. When fed to dairy steers, moulded hay was estimated to have about 70% the feed value of good quality hay. (29 references) M. O'LEARY.

Effects of level of intake and roughage content of cattle diets on digestibility, urinary energy, and mineral losses, and certain aspects of rumen function. W. D. C. Reed, R. C. Elliot and J. H. Topps (*Rhod. J. agric. Res.*, 1968, **6**, 81–91).—With increasing roughage content (5–50%) at three energy levels, and with protein, Ca, Mg and P at similar levels, digestibility fell as the roughage content increased, and was not affected by large increases in the energy value of the diet. In the rumen liquor, the % of volatile acids and the rate of CO_2 production were highest, and the pH lowest, after feeding the highest levels of the most digestible diets. The HOAc in rumen liquor was largely replaced by higher fatty acids after feeding on the better diets. Urinary energy losses increased with decreasing feeding levels. The highest levels of Mg and P were excreted after feeding high energy diets. (39 references.) P. S. ARUP.

Effect of level of feed intake and time of sampling after feeding on concentrations of dissolved sodium, potassium, calcium, magnesium and phosphorus in bovine rumen fluid. H. Fenner, F. N. Dickinson and H. D. Barnes (*J. Dairy Sci.*, 1969, **52**, 205–210).—Feeding trials with four rumen-fistulated cows fed on all-roughage ration showed that increased feed intake lowered the concn. of Na in the rumen fluid and raised those of Ca and Mg. Increase in feed intake initially increased K concn. but subsequently decreased it. Rate of intake affected P concn. in a very irregular fashion. Concn. changes as a function of time after feeding showed significant decreases for Na and increases for K and Mg during the first 4 h after feeding. Concn. returned to prefeeding levels at the second daily feeding. In the case of Ca and P, no definite trend in hourly concn. changes could be established. (17 references.) M. O'LEARY.

Transfer of nitrogen from the blood to the rumen in cattle. J. E. Vercoe (*Aust. J. agric. Res.*, 1969, **20**, 191–197).—In both a Brahman × Hereford and a Hereford steer fed on a chaffed tropical pasture hay, there was a limit to the amount of urea passing from the blood to the rumen of about 17–20 g of N/day, which was reached at plasma urea concn. of approx. 12 mg of N/100 ml. This concn. of plasma urea was achieved in the Brahman × Hereford cross when 23 g of N/day was infused intravenously but in the Hereford when 32–35 g of N/day was infused. (17 references.) E. G. BRICKELL.

Some effects of water restriction on nitrogen metabolism of cattle. R. F. Thornton and N. G. Yates (*Aust. J. agric. Res.*, 1969, **20**, 185–189).—Water restriction decreased N retention, the latter being associated with increases in both faecal N output and urinary urea excretion. The rise in urinary urea-N excretion appeared to be mediated through increased plasma urea-N concn. (23 references.) E. G. BRICKELL.

Effect of heat stress on energy and water utilisation of lactating cows. R. E. McDowell, E. G. Moody, P. J. Van Soest, R. P. Lehmann and G. L. Ford (*J. Dairy Sci.*, 1969, **52**, 188–194).—Experiments with lactating Holstein cows showed that maintenance requirements are considerably higher when the animals are under thermal stress. The results also suggested that in hot areas where protein shortages are acute there may be additional protein losses through the sweat glands. (24 references.) M. O'LEARY.

Effect of varying forage-to-concentrate ratio of isonitrogenous rations on feed intake by ruminants. R. L. Cowsert and M. J. Montgomery (*J. Dairy Sci.*, 1969, **52**, 64–67).—The results of experiments with Holstein heifers indicated that ruminants, while being fed isonitrogenous rations varying in energy concn., are capable of regulating the amount of food consumed so as to maintain a constant energy intake, provided fill or rumen load does not limit consumption. (13 references.) M. O'LEARY.

Pacific Northwest soft white wheat for lactating cows. R. G. McPherson and D. E. Waldern (*J. Dairy Sci.*, 1969, **52**, 84–89).—Lactation and acceptability trials showed that good results were obtained when high-producing dairy cows were fed large quantities of pelleted concentrates containing up to 93% of steam-rolled soft white wheat, with lucerne hay as sole roughage. (21 references.) M. O'LEARY.

Comparisons between alfalfa [lucerne] silage and hay. J. W. Thomas, L. D. Brown, R. S. Emery, E. J. Benne and J. T. Huber (*J. Dairy Sci.*, 1969, **52**, 195–204).—In six trials comparing direct-cut lucerne silage with lucerne hay, cattle and sheep consumed more dry matter as hay than as silage, but performance was not consistently greater with hay. Dry matter from silages contained more energy and ether extract and less hemicellulose than did the corresponding hay. The energy from silages was slightly more digestible than that from hay. (26 references.) M. O'LEARY.

Dry matter disappearance of roughages in nylon bags suspended in the rumen. M. W. Neathery (*J. Dairy Sci.*, 1969, **52**, 74–78).—Dry matter disappearance values of lucerne and Coastal Bermuda grass obtained by the nylon bag technique were similar to published data obtained by conventional digestion trials, but those of the higher fibre roughages of cottonseed hulls, corncobs, soyabean straw and maize stalks were considerably lower. Disappearance values were higher with a steer on a Coastal Bermuda grass diet than on lucerne–orchardgrass and were significant for all roughages except cottonseed hulls and maize stalks. A significant roughage–time interaction was noted, indicating that some roughages required a shorter time in the rumen than others to reach a max. disappearance. (11 references.) M. O'LEARY.

Comparative feeding value of wheat, corn [maize], barley, milo, oats and a mixed concentrate ration for lactating cows. R. S. Tommervik and D. E. Waldern (*J. Dairy Sci.*, 1969, **52**, 68–73).—Pelleted grain rations containing 95·7% of either wheat, maize, barley, milo, oats or a control mixed grain ration were compared in trials with dairy cows. The results indicated that, although there was some difference in acceptability (especially of maize) and in nutrient intakes, the six rations were similar in their effects on milk production when fed with lucerne hay at a 47 : 53 concentrate : roughage ratio. (16 references.) M. O'LEARY.

Effect of different lipids in the ration of lactating dairy cows on composition of milk. H. P. Adams, V. R. Bohman, A. L. Lesperance and J. M. Bryant (*J. Dairy Sci.*, 1969, **52**, 169–171).—The feeding of animal or plant sterols or a mixture of both had no significant effects on milk *SNF* or cholesterol levels. Animal sterol depressed *FCM* and both animal and plant sterols depressed fat test and milk fat production significantly. Feeding either tallow or vegetable oil depressed milk fat test significantly but significantly increased milk production, milk fat production, *FCM*, blood fat and blood cholesterol. (11 references.) M. O'LEARY.

Milk fat synthesis on restricted-roughage rations containing whey, sodium bicarbonate and magnesium oxide. J. T. Huber, R. S. Emery, J. W. Thomas and I. M. Yousef (*J. Dairy Sci.*, 1969, **52**, 54–59).—36 Holstein cows were fed concentrates *ad lib.* and 2·4 kg of hay/day for 8 weeks. The concentrates contained varying levels (0, 3, 7 and 14%) of whey, or minerals (2·5% $NaHCO_3$ and 1% MgO), or both whey (14%) and minerals. Fat depression, concentrate intake and milk yields were lowest on the mineral ration. Milk fat increased with increase in whey in the concentrate. Rumen pH was increased by the mineral diet but was not affected by whey. Minerals and whey decreased rumen propionate and increased rumen acetate. Rumen butyrate was increased only by 14% whey. Increased mammary uptake of fatty acids from heparin-precipitatable lipoprotein was directly proportional to arterial concn. and accounted for most of the increased milk fat synthesis on the mineral rations and for half the increase on whey. A larger uptake of β-hydroxybutyric acid occurred with 14% whey

than for the other rations and was associated with higher concn. of the C_6–C_{14} fatty acids in the milk. Milk stearate and oleate were increased by both minerals and whey but linoleate was decreased by additives. Stimulation of milk fat synthesis by whey and minerals is considered to occur by a different mechanism from that in operation with a normal ration sufficient in roughage. (18 references.) M. O'LEARY.

Effects of sodium bicarbonate, magnesium oxide and calcium hydroxide on milk fat secretion. J. W. Thomas and R. S. Emery (*J. Dairy Sci.*, 1969, **52**, 60–63).—The addition of 0·70–2·10% of $NaHCO_3$ plus 0·35–1·05% of MgO to grain mixtures fed to cows on a high grain, restricted roughage ration, increased milk fat % and daily secretion. There was a linear increase in fat % with increase in mineral levels. The middle level of minerals fed had the greatest effect on daily secretion. No differences in fat %, milk production or grain consumption were detected when the highest mineral level was administered directly to the rumen or mixed with the grain ration. This mineral level fed to cows on normal rations did not affect fat % but grain consumption decreased. $Ca(OH)_2$ was as effective as MgO in maintaining fat % but was less acceptable to the cows. M. O'LEARY.

Weight changes in lactating Holstein cows. R. H. Miller, N. W. Hooven, jun. and M. E. Creegan (*J. Dairy Sci.*, 1969, **52**, 90–94).—Body wt. and monthly milk yields from 1004 Holstein lactations were studied throughout the lactation. First-calf heifers gained the most and mature cows the least wt. during the lactation. Second and later gestations were marked by decreases in wt. from the first to the second month of lactation, whereas first-parity animals gained slightly during this period. This difference is attributed to the mobilisation of the fat reserves of older cows to meet lactation demands. (16 references.) M. O'LEARY.

Comparative nutritional value of liquid milk and dried milk for young calves. E. W. Swanson, J. E. Thigpen, J. Huskey and B. P. Hazlewood (*J. Dairy Sci.*, 1969, **52**, 228–234).—Feeding trials with Jersey calves showed that feeding of reconstituted skim-milk (**I**) gave significantly greater wt. gains than feeding of dried **I**. Metabolism tests showed that protein was digested better when **I** was fed in liquid form than when fed dry, but there was no significant difference in N retention. Average digestibility and retention of Ca and P were slightly higher for calves fed liquid **I** than for those given the dried form. (13 references.) M. O'LEARY.

Growth of young calves and rats fed soya flour treated with acid or alkali. B. M. Colvin and H. A. Ramsey (*J. Dairy Sci.*, 1969, **52**, 270–273).—Growth of calves and weanling rats fed either acid-treated or alkali-treated soya flour was superior to that of animals fed untreated flour. The reasons for this are unknown as no evidence of the presence in untreated flour of a pH-labile water-sol. growth inhibitor was obtained. M. O'LEARY.

Effects of induced mild hyperthyroidism on serum protein-bound iodine, thyroxine distribution volume and biological half-life of thyroxine-^{131}I in dairy cattle. T. R. Bauman, R. R. Anderson and C. W. Turner (*J. Dairy Sci.*, 1969, **52**, 245–249).—Lactating and dry dairy cattle were injected with exogenous L-thyroxine (**I**) at levels 25 and 50% in excess of the normal thyroid hormone excretion rate. The biological half-life of **I** decreased with increase in induced hyperthyroidism. Serum protein-bound **I** increased with increase in injected **I**. **I** distribution vol. increased over control values at 125 and 150% of normal thyroid secretion rate but values at the 150% level declined below those at the 125% level. Thyroid secretion rates obtained by the replacement technique and by the isotope dilution technique were in fair agreement, but the calculated utilisation rates of **I** greatly exceeded the daily injected dose of **I** at both 125 and 150% of normal thyroid secretion rate. (19 references.) M. O'LEARY.

Effects of iodine-131 thyroid damage on lactation and thyroid function in the bovine. J. K. Miller and E. W. Swanson (*J. Dairy Sci.*, 1969, **52**, 95–100).—The effects of partial thyroid destruction, produced by single oral doses of ^{131}I varying from 99 to 180 μCi/kg of body wt., were studied in trials with nine pairs of identical twin dairy heifers. First-calving reproduction was normal in thyroid-damaged heifers. Thyroid I uptake, thyroxine secretion rate, plasma protein-bound I and heart rate were markedly reduced by ^{131}I thyroid damage. Average measurements for control and thyroid-damaged heifers, respectively, were: milk yield 4000 and 2042 kg; milk fat, 4·29 and 3·96%; *SNF*, 8·77 and 8·66%; 4% fat-corrected milk, 4186 and 2032 kg. (13 references.) M. O'LEARY.

Effect of physiological status on diet selection by grazing ewes. W. R. McManus, G. W. Arnold and J. Ball (*J. Br. Grassld Soc.*, 1968, **23**, 223–227).—The dietary selection by dry ewes grazing pastures over a range of availabilities was compared with that of ewes in various stages of pregnancy and lactation using oesophageal fistulae. There were no significant differences at any stage of reproduction in the botanical composition of the selected diets. Small differences in digestibility, sol. carbohydrate and N% of the diets were found during pregnancy and lactation, and this occurred at all levels of availability of pasture. Dry fistulated ewes can be used to obtain samples for assessing dietary N and sol. carbohydrate content for ewes in other physiological states. A. H. CORNFIELD.

Summer nutrition of immature sheep: nitrogen excretion of grazing sheep in relation to supplements of available energy and protein in a Mediterranean environment. W. G. Allden and A. C. Jennings (*Aust. J. agric. Res.*, 1969, **20**, 125–140).—Unsupplemented sheep grazing mature pasture herbage excreted 23·4 g of N/day in urine and faeces. Supplementary energy produced an N-sparing effect whereas added protein was associated with an increased excretion of N, particularly in the urine. No significant changes in estimated herbage intake were associated with changing N but significant increases in wool production were associated with supplementary N. Intakes of sheep grazing the mature herbage of sown clover-grass pastures in Southern Australia were commonly of the order of 12–18 g of N/day. (37 references.) E. G. BRICKELL.

Effect of one and two days *ante mortem* fasting on live weight and carcass losses in lambs. A. H. Kirton, A. R. Quartermain, A. E. Uljee, W. A. Carter and F. S. Pickering (*N.Z. Jl agric. Res.*, 1968, **11**, 891–902).—Fasting (48 h) resulted in loss of 4 kg live wt. and 0·57 kg carcass wt. (mainly water). No carcass loss resulted from the first 24 h fasting. The different treatments caused no differences in the palatability of the meat. The availability of drinking water did not affect any of the observed fasting losses. (15 references.) E. G. BRICKELL.

Influence of diet composition on the utilisation of soft phosphate in broiler diets. B. L. Damron, P. W. Waldroup and R. H. Harms (*Poult. Sci.*, 1967, **46**, 1544–1549).—Experiments were conducted to determine factors influencing growth of broilers while using soft phosphate as the sole source of supplementary P; a range of supplementary Ca was provided by powdered limestone. Four-week body wt. equiv. to positive control diets could not be achieved with diets containing soft phosphate as the sole source of supplementary P. Addition of 2·5% of fish meal improved 4-week body wt. but not to the extent of birds having the control diet containing 0·8% Ca. Addition of 0·4% supplementary P and 0, 0·1 or 0·2% supplementary Ca, together with 2·5% fish meal resulted in 8-week average body wt. statistically equal to the wt. of the control group. A. H. CORNFIELD.

Effect of different levels of dehydrated alfalfa [lucerne] meal in practical rations of laying hens on pigmentation of egg yolk: their influence in utilisation of red xanthophylls. F. Tortuero (*Poult. Sci.*, 1968, **47**, 376–383).—The xanthophylls of dehydrated lucerne meal, added at 3–6% in the hen's diet, had little influence on egg yolk pigmentation. Addition of red xanthophylls (capsantin and capsorrubin) at 3–30 g per 1000 kg of diet resulted in increased pigmentation of egg yolk. Even though the xanthophylls in lucerne had small pigmenting power, their presence in the hen's ration resulted in increased pigmentation when red xanthophylls were also added. A. H. CORNFIELD.

Influence of soyabean products on the bone ash of chicks fed phosphorus-deficient diets. M. Griffith (*Poult. Sci.*, 1968, **47**, 765–771).—Replacing purified cellulose with coarsely ground soyabean hulls or soyabean meal in a purified P-deficient diet increased the bone ash in chicks. The response to the soyabean products disappeared when these were ground to a fine powder before addition to the diets. The antirachitic effect of these materials is due to physical properties. A. H. CORNFIELD.

Influence of steam pelleting on the nutritional value of chick diets. H. S. Bayley, J. D. Summers and S. J. Slinger (*Poult. Sci.*, 1968, **47**, 931–939).—Steam pelleting (70–90°) of 24%-protein maize–soyabean meal and wheat–soyabean meal diets improved the performance of chicks to a greater extent than did steam pelleting of a 17%-protein maize–soyabean meal diet. When the soyabean meal added to the low-protein diet was pelleted beforehand chick performance was poorer than with the control diet. Steam pelleting and regrinding wheat produced a greater improvement than did pelleting and regrinding maize. Results were similar whether pelleting temp. of 70, 80, or 90° were used. The effects of pelleting on chick performance could not be explained on the basis of

changes in metabolisable energy of the diets resulting from the treatments. A. H. CORNFIELD.

Autoclaving time in relation to the nutritional quality of dried egg white for chicks. M. Kelly and H. M. Scott (*Poult. Sci.*, 1968, **47**, 850–852).—When dried egg white was added to a purified diet as the sole source of protein it was found that autoclaving (121°) for more than 5 min. before addition to the diet severely depressed wt. gains and feed efficiency of chicks. Autoclaving for 1 h or longer depressed wt. gains almost to zero. A. H. CORNFIELD.

Calcium, phosphorus, and vitamin D_3 levels and interactions in turkeys to four weeks of age. L. H. Neagle, L. G. Blaylock and J. H. Goihl (*Poult. Sci.*, 1968, **47**, 174–180).—Three factorial experiments with dietary P levels of 0·4–1·0%, dietary Ca of 0·4–2·0% and vitamin D_3 levels ranging from 138 to 7700 I.C.U. per kg were tested with male turkeys to 4 weeks of age. Max. bird performance (wt. gains, feed efficiency, and toe ash %) was obtained with a diet containing 0·8% P, 1·2% Ca, and 550–1100 I.C.U. of vitamin D_3 per kg of diet. Providing dietary Ca was maintained at 0·8%, near max. performance was obtained with diets having 0·6% P. A. H. CORNFIELD.

Alkaloids as a possible cause of ryegrass staggers in grazing livestock. A. J. Aasen, C. C. J. Culvenor, E. P. Finnie, A. W. Kellock and L. W. Smith (*Aust. J. agric. Res.*, 1969, **20**, 71–86).—A high alkaloid content occurs consistently in the seedlings and new shoots of *Lolium perenne* L. which comprise the sole pasture of sheep and cattle subject to outbreaks of staggers. In mature grass the alkaloid is nearly all perloline (**I**), but in seedlings halostachine (**II**) may also be present as a major constituent. Parental administration of **I** to guinea-pigs and sheep produced staggers-like symptoms at 30 mg/kg (which could be attained in sheep grazing toxic grass). Oral administration of **I** at 100–200 mg/kg produced the same effects in guinea-pigs but not in sheep. **II** produced only mild effects in sheep and guinea-pigs at similar doses. **I** and **II** together, given either simultaneously by the oral route or in sequence (**II** intravenously followed by **I** orally) did not lead to disturbances resembling staggers. (34 references.) E. G. BRICKELL.

Animal haematology at high altitudes: haemoglobin and haematocrit changes. A. H. Javed and L. E. Washburn (*W. Pakistan J. agric. Res.*, 1968, **6**, 154–159).—Observations were made on the blood of rats, sheep and cattle (total 213) at altitudes of 3500–11,500 ft. In comparison with animals at low altitudes, there was an increase of > 8% in the haemoglobin per red blood cell of all the animals, and an increase of ~ 27% in the haematocrit of the blood of the sheep, but not in that of the cattle. (26 references.) P. S. ARUP.

Determination of lactose in blood plasma. H. de Langen (*N.Z. Jl agric. Res.*, 1968, **11**, 816–820).—Lactose in bovine plasma is separated from glucose and other interfering material by adsorption on charcoal and determined colorimetrically with phenol in the presence of H_2SO_4. (10 references.) E. G. BRICKELL.

Cattle lice. Anon. (*Leafl. U.S. Dep. Agric.*, 1969, No. 456, 8 pp.).—Control of *Linognathus vituli*, *Haematopinus eurysternus*, *Solenopotes capillatus* and *Bovicola bovis* by insecticidal sprays, dips and dusts is described. E. G. BRICKELL.

Ultra-low volume application of insecticides to cattle for control of horn fly. J. L. Eschle and A. Miller (*J. econ. Ent.*, 1968, **61**, 1617–1621).—An automatic sprayer is described and illustrated. The cost of its use compared very favourably with that of conventional dust application. In field tests 1% Ciodrin and 1% malathion applied by the sprayer controlled horn flies on dairy and beef cattle, respectively, for the summer season (1966). (20 references.) C. M. HARDWICK.

Field experiments with attractants for the face fly. C. K. Dorsey (*J. econ. Ent.*, 1968, **61**, 1695–1696).—Synthetic and natural animal and plant products were tested in petri dishes (placed along the edge of a pasture) and on steers. The attractants were more effective when applied on the animals. (13 references.) C. M. HARDWICK.

Residues of Gardona in body tissues of cattle sprayed to control *Hypoderma* (spp.). M. C. Ivey, R. A. Hoffman and H. V. Claborn (*J. econ. Ent.*, 1968, **61**, 1647–1648).—Cattle were sprayed with 0·125, 0·25 and 0·5% concn. of Gardona [2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate]. Almost all residues were in fatty tissue. The max. was 0·1 ppm after 1 week and there were no residues after 3 weeks. C. M. HARDWICK.

Chronic toxicity to laying hens and degradation of Bayer 18779 (*O*-ethyl-*O*-isopropyl-*O*-phthaloximido- phosphorothioate. M. Sherman, G. H. Takei, R. B. Herrick and E. Ross (*Poult. Sci.*, 1968, **47**, 648–654).—Addition of Bayer 18779 at 100 ppm for 36 weeks followed by 200 ppm for another 13 weeks to the diet of laying hens had no significant effect on mortality, body wt., feed consumption, egg production or other egg characteristics, but reduced feed efficiency. Blood plasma cholinesterase activity was inhibited initially by the treatment, but this inhibition decreased with age of feed mixture. Bayer 18779 applied to the feed of laying hens was too unstable to effect a high level of protection against the breeding of maggots in the droppings. (11 references.) A. H. CORNFIELD.

Effects of supplying amprolium to hens on amprolium levels in the egg yolk. D. Polin, W. H. Ott, E. R. Wynosky and C. C. Porter (*Poult. Sci.*, 1968, **47**, 795–799).—When the coccidiostat amprolium (**I**) [1-(4-amino-2-n-propyl-5-pyrimidinylmethyl)-2-picolinium chloride] was supplied to hens in the feed or water detectable concn. of **I** was found in the yolks after 2–3 days, and max. concn. 6 days afterwards. When supplied at 240 ppm in the feed or water, yolks contained 1·15 ppm when **I** was added to the feed and 3·9 ppm when added to the water. There was a linear relationship between the log concn. of **I** in the diet (62·5–20,000 ppm) and that in the yolk. Yolks no longer contained detectable amounts of **I** (< 0·16 ppm) 8–10 days after termination of administration of the drug. A. H. CORNFIELD.

Atomic absorption spectrophotometric and ethylenediaminetetra-acetate titration methods for calcium and magnesium determinations. J. Lee and C. M. Campbell (*J. Dairy Sci.*, 1969, **52**, 121–124).—The EDTA titration and atomic absorption spectrophotometric methods for determining Ca and Mg were compared using solutions of known concn. and subtropical forages as test media. The results showed that the atomic absorption method is faster and more accurate than the EDTA titration method. (10 references.) M. O'LEARY.

Microdetermination of the drug ingredients 2-chloro-4-nitrobenzamide, 4′-[*p*-nitrophenyl)sulphamoyl]acetanilide and 4-aminobenzenearsonic acid in medicated finished feeds. M. Malaiyandi, S. A. MacDonald and J. P. Barrette (*J. agric. Fd Chem.*, 1969, **17**, 51–55).—Microamounts (100–1000 μg/g of feed) of the title compounds (aklomide, **I**, sulfanitran, **II**, and *p*-arsanilic acid, **III**, respectively) were determined by separating **I** and **II** from **III** by t.l.c. and estimating **I** and **II** individually by colorimetry using the Bratton–Marshall reagent. The determination was repeated after alkaline digestion of a sample of the feed, giving total **I** + **II** concn., from which the **III** content could then be calculated by difference. Recoveries of **I** and **III** were > 96% and ~ 90%, respectively. (13 references.) P. S. ARUP.

Residues of fenthion and five of its metabolites: their persistence in corn [maize] and grass forages. D. B. Leuck and M. C. Bowman (*J. econ. Ent.*, 1968, **61**, 1594–1597).—Sprays of fenthion were applied at 4 lb/gal and residues were determined over 21 days. On maize, fenthion was mostly metabolised to fenthion sulfoxide. After 14 days all residues were < 1 ppm. Similar results were obtained with Coastal Bermuda grass and residues were < 1 ppm after 21 days. When the maize was ensiled in a jar, 1 day after spraying, residues oxidised more slowly. C. M. HARDWICK.

Effects of feeding cattle forage treated with Mobam. M. C. Bowman, R. S. Lowrey, D. B. Leuck and M. Beroza (*J. econ. Ent.*, 1968, **61**, 1495–1499).—Coastal Bermuda grass was treated with 0·5–2 lb/acre of Mobam (benzo[*b*]thien-4-yl methylcarbamate) (**I**) and residues were analysed by g.c. Values dropped by 10% between application and ensiling and by 13–27% during the next 36 days. Residues were then 8·5–27 ppm and did not alter for the next 30 days. Milk produced by cows fed on silage containing up to 90 ppm of **I** on a dry-wt. basis contained no detectable residues. There was no sign of decreased blood cholinesterase levels. (19 references.) C. M. HARDWICK.

Excretion and storage of dieldrin in dairy cows fed thyroprotein and different levels of energy. D. G. Braund, L. D. Brown, J. T. Huber, N. C. Leeling and M. J. Zabik (*J. Dairy Sci.*, 1969, **52**, 172–182).—Experiments with Holstein cows and heifers showed that neither alteration in energy uptake nor thyroprotein feeding are of practical value for decontaminating dairy cows that are contaminated with dieldrin. (13 references.) M. O'LEARY.

Metabolic studies with Alar growth regulator in the dairy cow. L. E. St. John, jun., H. Arnold and D. J. Lisk (*J. agric. Fd Chem.*, 1969, **17**, 116–117).—None of the Alar [succinic acid mono(2,2-dimethyl hydrazide)] fed to a cow at 25 ppm of the feed passed into the milk; about 93% was recovered as such in the faeces and

the urine. Alar was stable towards fresh rumen fluid, fresh beef liver and the liver homogenate. P. S. ARUP.

Animal feed supplement. Wenner-Gren Medical Laboratory A.-B. (B.P. 1,134,206, 28.1.66. Swe., 1.2.65).—An animal feed supplement containing a growth promoting additive comprises lactic acid-producing yoghurt bacteria (*Bacillus bulgaricus* or *Streptococcus thermophilus*) in the form of a dry powder containing viable bacteria; between 0·1 and 5·0(1–4)% is added to the feed. In an example the bacteria are cultured at 42° for 2–5 h in skim-milk and then cooled to 15° and spray dried at 60–65°. Swine fed on a commercial feed containing the additive showed increased wt. gain and gave higher quality products. S. S. CHISSICK.

Food composition for feeding ruminants. Commonwealth Scientific and Industrial Research Organisation (B.P. 1,137,214, 20.6.67. Australia, 21.6.66 and 1.5.67).—Protein and amino acid feeds/feed supplements for increasing protein production relative to feed intake in ruminants are claimed, whereby body growth, meat, milk and/or wool production are increased. Proteinaceous feed particles are chemically modified and/or coated to form a polymeric substance or complex stable at pH > 5 and unstable at pH <4, so that it is resistant to microbial attack in the rumen, but is digested in the abomasum and small intestine. This substance is produced, e.g., by treatment of the protein with an aldehyde or it is a coating of a vinyl or acrylic polymer. In an example commercial HCl-pptd. casein (< 30 mesh) is stirred with aq. HCHO, separated, washed and dried. On incubation with rumen concentrate *in vitro* at 39·5° the treated casein is virtually unattacked.
S. S. CHISSICK.

Improvement in poultry feeding. Takeda Chemical Industries Ltd. (B.P. 1,138,340, 29.12.65. Jap., 29.12.64).—A method for raising the rate of egg production, the fertility and hatchability, comprises feeding the poultry on a conventional feedstuff to which has been added (5–100 mg/kg of feedstuff) at least one compound of general structure thiamine-R-disulphide, where R is Pr, Bu, heptyl, tetrahydrofurfuryl or tetrahydropyranyl-2-methyl.
S. S. CHISSICK.

Treatment of hypocalcaemia. National Research Development Corp. (Inventor: R. A. Gibbons) (B.P. 1,137,540, 20.12.66).—Calcium deriv. (**I**), especially useful in the treatment of, e.g., cows during parturition, are claimed. **I** are neutral deriv. of high mol. wt. polysaccharides (**II**), e.g., starch, cellulose or dextran, in which 60–90% of the monosaccharide rings are open and oxidised to CO_2H groups (present as Ca and Na salts) such that the product contains ~7% of Ca by wt. Prep. is by oxidation of **II**, using first periodate and then chlorite, followed by neutralisation with a mixture of Ca and Na salts. Physiologically unacceptable anions are removed by dialysis against distilled water. S. S. CHISSICK.

Isothiocyanato derivatives. Agripat S.A., Assee of F. Paltauf and A. Margot (B.P. 1,136,760, 29.12.65. Switz., 30.12.64).—Compounds of formula $C_6H_3RR^{III}\cdot NCS$ are anthelmintic agents of reduced toxicity to animals, wherein R^{III} is H, halogen, OH, NO_2, CN, CNS, NCS, CO_2H, carbalkoxy, SO_2NH_2, alkyl of 1–9 C, alkoxy, halogenoalkyl, dialkylamino, alkyl- or dialkyl-sulphamyl, alkylsulphonamido, halogenoalkyl- or aryl-sulphonamido, or alkyl-sulphonyl or -sulphinyl; R is $X\cdot C_6H_3R^IR^{II}$ or $Y\cdot R^{IV}$; X is O or S; Y is SO or SO_2; R^I and R^{II} are similar to R^{III}; and R^{IV} is Ph which may contain 2 halogen or 2 NCS, or 1–2 other groups represented by R^{III}. An example is di-(*p*-isothiocyanatophenyl) ether, m.p. 67·5–68·5°. It is prepared by adding aq. suspension of $O(C_6H_4NH_2\text{-}p)_2$ during 80 min. to an aq. emulsion of $CSCl_2$ at 10–12°, then working up after 2 h at room temp. The yield is 83·4%. F. R. BASFORD.

2.—FOODS

Carbohydrate Materials

Cereals, flours, starches, baking

Free protein and free starch in wheat and rye flour. M. Rohrlich and V. Müller (*Stärke*, 1969, **21**, 29–38).—Commercial wheat and rye flours were subjected to gravity sedimentation using a CCl_4/C_6H_6 medium of sp. gr. 1·45. The starch separated in this manner contained 2–3% protein from which gluten could not be obtained. Up to 50% of the sedimented protein was water-sol. and gel filtration on G 200 Sephadex indicated a mol. wt. distribution from 10,000 downwards. It showed high proteolytic activity with haemoglobin as substrate. Apart from the glutamic acid, the amino acid composition of this protein fraction was distinctly different from the reserve protein which contained large amounts of the basic amino acids. Because of these properties it is considered to be starch albumin. The free starch exhibited swelling and gelatinisation properties which differed from those of adhesive starch, and the max. viscosity depended on the protein content. The two types of flour differed in their free starch contents. (12 references.) J. B. WOOF.

Rate of moisture adsorption by wheat flour and its relation to physical, chemical and baking characteristics. K. H. Udani, A. I. Nelson and M. P. Steinberg (*Fd Technol., Champaign*, 1968, **22**, 1561–1564).—Air at various const. R.H. (40–80%), at 21°, was passed over flour samples in tubes, and the moisture adsorption was determined by periodic weighings. The adsorption rate, calculated from the exponential time–wt. curves, was directly proportional to the R.H., but inversely proportional to the protein content of the flour; it was not related to particle size or cake vol. (26 references.) P. S. ARUP.

Effect of manufacturing conditions on the properties of phosphate-modified maize starches and their application. W. Nierle (*Stärke*, 1969, **21**, 13–18).—In spite of the wide application of modified starches in the food industry, where it is possible to vary the properties of the product by modifying production conditions, it is not yet possible to predict the effects of heating temp., phosphate content, pH or multiple esterifications on the final η. Several phosphated starches were examined in a Brabender viscograph. The amount of Na tripolyphosphate used in the prep. had little effect. Max. η was reached at 150° and at pH 9·5 where the bound phosphate reached 0·38% (0·18% at pH 8·5). The effects of repeated reaction at 140° were confined to the first η max. after the initial increase. The behaviour of Na mono- and di-phosphates of starch prepared from Na trimetaphosphate is discussed. (34 references.) J. B. WOOF.

Effects of gamma irradiation on some technological characteristics of maize for starch production. Comparison with drying at high temperature. L. Saint-Lèbe (*Stärke*, 1969, **21**, 8–13).—Moist maize was treated at 200, 400 and 1000 krad using ^{60}Co γ-radiation and the air-dried product was compared with that produced by drying at 74°. Irradiation up to 400 krad had no effect on the extractability of the starch on a large scale or on its degradation with α-amylase. The treatment improved gelatinisation and lowered the viscosity of the paste by 25% at 200 krad. These effects are attributed to depolymerisation of the starch and weakening of the grain hull. Hot air drying caused greater and irreversible changes in the maize. (18 references.) (In French.)
J. B. WOOF.

Determination of carotenoids in bakery products and their raw materials. I. Wildfeuer and L. Acker (*Mitt. Geb. Lebensmittelunters. u. Hyg.*, 1968, **59**, 392–400).—The total carotenoids extracted with EtOH–C_6H_6 from 39 samples of durum semolina and flour and determined by spectrophotometry at 446 nm in Me_2CO solution amounted to 3·6–6·2 (average 5·3) mg/kg. After evaporation of the Me_2CO, the carotenoids were dissolved in light petroleum and submitted to column chromatography on Al_2O_3 with light petroleum as eluent. The comparatively rapidly-moving β-carotene amounted to only ~1% of the total carotenoids, most of which remained near the top of the column. Additions of commercially stabilised carotenoids such as ethyl β-apo-8-carotinoate, β-apo-8-carotenal and canthaxanthin could be detected down to 2 mg/kg. (12 references.) P. S. ARUP.

Ingredient effects on meringues cooked by microwaves and by baking. R. E. Baldwin, R. Upchurch and O. J. Cotterill (*Fd Technol., Champaign*, 1968, **22**, 1573–1576).—In both cooking methods, guar gum (0·6%) was the most efficient ingredient for the prevention of sagging and the loss of liquid. Triethyl citrate (0·03%) plus Na lauryl sulphate (0·03%) decreased whipping time and increased whipped vol. Algin (0·6%) was the least satisfactory ingredient. No advantage was gained by altering the pH of the mixture. Electronically cooked meringues were whiter and more compact in texture than baked meringues. (13 references.) P. S. ARUP.

Method of treating rice. Kyowa Hakko Kogyo K.K. (B.P. 1,134,793, 4.7.67. Jap., 4.7.66).—A process is claimed for preparation of polished rice fortified with amino acid (**I**), which has a reduced tendency to discolour on storage and improved resistance to loss of **I** on washing with water. The rice, optionally pretreated with steam, etc., is first coated with a mixture of a **I**, e.g., L-lysine,

and a Ca^{2+} salt (chloride, carbonate, lactate or acetate) in the ratio 10^3 : 1–20 and then further coated with non-toxic, water-sol. film, forming agent (Ca/Na cellulose glycollate). About 10% of **I**, based on rice, and ~ 0·1–2% of Ca salt, based on **I**, are used.
S. S. CHISSICK.

Preservation of bakery products. Farbwerke Hoechst A.-G. (B.P. 1,134,302, 20.1.67. Ger., 20.1.66).—There is incorporated in the dough, prior to baking, 0·01–3 (0·1–1·5) wt.-% of a mixed anhydride of sorbic acid and an aliphatic carboxylic acid (> 5 C, e.g., a mixture of 12–18 C fatty acids produced from natural fats and oils) or a mixture of two or more such mixed anhydrides.
J. M. JACOBS.

Manufacture of granular [baker's] yeast and packs containing it. Distillers Co. (Yeast) Ltd. (Inventor: T. Beckett) (B.P. 1,135,418, 24.11.66).—A free-flowing, granular yeast suitable for packaging, a package for containing the yeast and a method for its manufacture, are claimed. An aq. suspension of yeast is concentrated until it is creamy and then treated with ≮ 1·5 (2–2·5)% wt./vol. of an osmotic agent, e.g., NaCl. After a few minutes at 34–50°F the yeast is separated, washed with water and dried to ≯ 31% of the original wt. The yeast is subdivided, sieved and packaged in, e.g., a polyethylene container which is sealed with a one-way valve.
S. S. CHISSICK.

Sugars and confectionery

Formation of sucrose pyrolysis products. R. R. Johnson, E. D. Alford and G. W. Kinzer (*J. agric. Fd Chem.*, 1969, **17**, 22–24).—Volatiles formed by sucrose pyrolysis were compared with those formed on boiling a solution of glucose in an acid solution of $SnCl_2$. Out of 26 compounds, 12 were shown by g.l.c., i.r. spectrometry and mass spectrometry to be formed by both processes. Three new products of pyrolysis were found, 2-methyl-2-cyclopenten-1-one, γ-butyrolactone and 5-methyl-2,5*H*-furanone. P. S. ARUP.

Process technology in the chocolate industry. E. Sann (*Chemie-Ingr-Tech.*, 1969, **41**, 352–358).—A discussion of the manufacture of chocolate goods deals with the pretreatment of the cocoa beans in the producer countries, the production of cocoa mass, butter, powder and chocolate and finishing of the products. Flow sheets of the most important machines such as roasters and tempering plants are presented. The operations of roasting, breaking, purification, size reduction, blending, pre-crystallisation, casting into bars and coating and packaging are described. The training of personnel is also discussed. (14 references.)
M. SULZBACHER.

Syrups of low dextrose equivalent. A. E. Staley Mfg Co. (Inventor: T. L. Hurst) (B.P. 1,133,914, 22.6.67. U.S., 14.7.66).—A method is claimed for the dual hydrolysis of starch whereby starch conversion syrup of low (22–30) D.E., and stable to haze formation is obtained. An acidified aq. starch dispersion is (*i*) acid-hydrolysed by heating to a D.E. of 13–16 and (*ii*) cooled and enzyme-hydrolysed by treatment with α-amylase (**I**). After deactivating **I** the hydrolysate is filtered and concentrated. In an example a starch slurry is adjusted to pH 1·85 with HCl and it is heated: (*a*) at 190°F with agitation for 30 min and then (*b*) at 210–265°F in an autoclave for 7·5 min. After adjustment of the autoclaved liquid to pH 5·7, **I** is added and the temp. maintained (*c*) at 170°F for 7 h and then (*b*) at 200°F for 15 min. The liquor is finally cooled, filtered and concentrated. S. S. CHISSICK.

Fermentation and Alcoholic Beverages

Continuous thin-layer fermentation. G. Gorbach (*Mschr. Brau.*, 1969, **22**, 49–52).—One of the principal disadvantages of batch fermentation procedures is the difficulty of ensuring that a continuous and adequate supply of nutrient is in contact with, and available to, the fermenting organism. The procedure and equipment necessary for thin-layer fermentation is described. The inoculated nutrient solution is run into a series of pipes to about one third of their total vol. and thin layers of liquid are formed continuously by rotating rollers which are mounted in the centre of the pipes. The character of the layers formed and the degree of aeration can be controlled by the design of the rollers and the speed of rotation. In this way, metabolic products are removed from the immediate neighbourhood of the cell promoting optimum metabolic activity. The technique is suitable for continuous operation and automatic control. Some preliminary growth studies on yeast are discussed. J. B. WOOF.

Flocculation of brewing yeast—A review. S. Windisch (*Mschr. Brau.*, 1969, **22**, 69–72).—A review is given of the following aspects of flocculation in yeasts used in brewing: genetics, flocculation in relation to the state of the fermentation, effect of charge, yeasts of differing flocculation type, structure and function of the cell membrane and the method of selection. (56 references.) J. B. WOOF.

Behaviour of bottom fermenting culture yeasts. C. C. Emeis (*Mschr. Brau.*, 1969, **22**, 72–75).—Top fermenting yeasts tend to stick to the walls of the tun near the surface of the wort, so that if the yeast in the laboratory or propagation plant is drawn from this region, the culture quickly becomes enriched with top fermenting strains. In order to avoid fermentation difficulties caused in this way, care should be taken to use a representative portion of the yeast for reinoculation and to sterilise the culture plant efficiently.
J. B. WOOF.

Effects of ripening, storage and variety on the water sensitivity of barley. L. Reiner (*Brauwissenschaft*, 1969, **22**, 47–53).—The effects on water sensitivity of the growth, harvesting, handling and storage conditions of Amsel, Volla, Wisa, Union, Donaria and Proctor barleys were investigated. For grain grown in 1965 and 1966, water sensitivity varied considerably with variety as a result of differing conditions during ripening. Late sowing tended to increase water sensitivity, but the level of N fertiliser used or accidental damage during threshing had little effect. Storage at 20° resulted in less water sensitivity than storage in the cold.
J. B. WOOF.

Germination tests on barley varieties harvested at different times and subjected to different treatments. M. Jochimsen (*Brauwissenschaft*, 1969, **22**, 42–47).—Samples of Union, Bido, Èli, Donaria and Proctor barleys grown in 1965–1967 were harvested at different stages of ripening. After different treatments, the changes in germinative ability during storage were followed. In general, the germinative ability increased with ripeness of the corns, but adverse weather conditions shortly before cutting were deleterious. After 5 months storage, there were no significant differences between the germination of grains harvested at full ripeness and that taken just before this stage. The storage conditions were (*i*) in air at 16% moisture, (*ii*) in air at 12% moisture, (*iii*) dried to 20% and then carefully to 16% at 50–60°, and (*iv*) hermetically sealed at 20% moisture and 25° for 10 days, then in air at 16% moisture. Method (*ii*) was generally most favourable and (*iv*) least satisfactory, though harvesting conditions could considerably affect storage behaviour.
J. B. WOOF.

Genetic regulation of enzyme synthesis in germinating barley by gibberellic acid and its importance in brewing. A. Piendl (*Brauwissenschaft*, 1968, **21**, 453–464).—The chemical structures and physiological functions of gibberellins and other plant hormones are discussed. The bases on which gibberellic acid (*GA*) influences the genes are listed and possible complex formation between *GA* and a repressor (histone) is described. The genetic regulation of α-amylase and protease synthesis by *GA* could occur at the following levels: RNA-polymerase, messenger RNA, interaction of messenger-, ribosomal- and transfer-RNA and formation of peptide chains. Probably a combination of all four routes is responsible. Barleys rich in gibberellins yield malts and worts with a high level of fermentable sugars, assimilable amino acids, nucleotides and phosphates, which have a favourable effect on the pattern of fermentation and character of the beer. (144 references.)
I. DICKINSON.

Investigation of heat formation during germination of barley using a bomb calorimeter. H. Kieninger (*Brauwissenschaft*, 1969, **22**, 6–13, 62–69, 100–104).—A reproducible micro-malting technique is described in which the loss in wt. of the barley during germination can be estimated with an accuracy of 0·1%. The malt is dried to a moisture content of 8–16%, ground and transferred to a bomb calorimeter with an analytical accuracy of 0·13%. The enthalpies determined for barley, germinating grain and malt were ~ 9·1% higher than those calculated from the fat, protein and carbohydrate contents, since in this case the chemically-bound energy, including that stored in org. phosphates, was unaccounted for; with progressive modification of the endosperm during germination, the degradation of energy-rich to energy-poor substances occurred. (60 references.) J. B. WOOF.

Semi-scale brewing trials using new raw materials and unconventional procedures. F. Schur, F. Ullmann, E. Schlienger and H. Pfenninger (*Brauwissenschaft*, 1968, **21**, 413–424).—Six beers were prepared in a 5 hl pilot brewery. Three unhopped worts were derived from a conc. enzymic extract of barley, and the other three were prepared from malt by mashing in the normal manner. One

of the unhopped worts from each source was left unboiled, another one was boiled before fermentation and the resulting beers were treated by 'cold hopping' with the pre-isomerised hop extract 'Redihop'. The wort prepared from barley extract or from malt was boiled with whole hops. The pitching worts, green and final beers were analysed; defects were apparent in the beers prepared with the new raw materials examined in the trials. These defects, particularly poor organoleptic qualities, are probably due to the low content of α-amino N in the barley extract and to the undesirable high content of unchanged hop oil in the 'Redihop'. (36 references.) I. DICKINSON.

Use of gel filtration in the study of biochemical changes which occur during brewing. G. Basařová (*Int. Brewers' J.*, 1969, **105**, 48–50).—The content of solids of different mol. wt. and groups in Pilsner worts and beers at various stages of brewing were determined by means of column chromatography on Sephadex and on Biogel. With the system of calculation described, mol. wt. were determined with an accuracy of ± 10%. Analytical and organoleptic results obtained with products of different brewing systems (batch, continuous and semi-continuous) were compared, as were also the enzymic activities during two- and three-mash boiling processes, and the effects of various beer stabilisers. The greatest losses of solids (mol. wt. 2000–8000) occurred during fermentation. Cooling generally removed complexes of ingredients with mol. wt. 20,000–70,000. P. S. ARUP.

Isolation and characterisation of panose and isopanose from wort and beer. G. N. Bathgate (*Chemy Ind.*, 1969, 520–521).—The conc. and electrodialysed beer or wort was chromatographed on Whatman No. 17 paper with Pr^nOH–EtOAc–H_2O (14 : 2 : 7) as solvent system to separate oligosaccharides according to their degrees of polymerisation. The trisaccharides were then rechromatographed on Whatman No. 3 paper for > 48 h in EtOAc–pyridine–H_2O (10 : 4 : 3) to yield a panose fraction and a maltotriose fraction. The panose fraction was further resolved by electrophoresis for 2 h on Whatman No. 1 paper in 0·1 M-$Na_2B_4O_7$, at 4° and 50 V/cm, to yield panose (M_G 0·42) and isopanose (M_G 0·67). These two compounds were differentiated by their degradation patterns on hydrolysis with dil. glucamylase solution at 30°; panose was slowly hydrolysed at the α-1,6-linkage to form glucose, whilst isopanose hydrolysed rapidly at the α-1,4-linkage to form glucose and isomaltose. Both compounds in the wort were probably produced either during malting or mashing, but were not synthesised by transglycosidation during fermentation. W. J. BAKER.

Influence of formaldehyde on malting. J. Riemann (*Brauwissenschaft*, 1969, **22**, 81–87).—Germinating barley was sprayed daily with a 0·1% solution of HCHO, by adding it to the steep liquor at 0·01% or by resteeping in water and then in 0·1% HCHO and subsequently spraying with a 0·1% solution. In each series, the levels of treatment were varied. All the forms of treatment had a distinct effect on the enzyme process essential for modification. Enzymes were inhibited generally, with the effect on proteolytic enzymes being most marked because of the decreased accessability of the substrate. Low hot water extracts were obtained which contained considerable amounts of incompletely degraded polysaccharide so that viscosities were high and attenuation limits low. The beneficial effects were diminished rootlet growth and malting loss and worts low in anthocyanogen, protein and colour. Suitable choice of levels of treatment can yield the advantages whilst minimising the disadvantages, resulting in a net improvement in quality and cost. (15 references.) J. B. WOOF.

Determination of formaldehyde in beer. K. Steiner, F. Schur and H. Pfenninger (*Brauwissenschaft*, 1969, **22**, 87–90).—HCHO added to beer reacts with protein, polyhydroxyphenols and alcohols (when its concn. is > ~3%). The levels measured by the chromotropic acid method must therefore be corrected. Degassed beer with antifoam is distilled and a portion of the distillate mixed with an equal vol. of chromotropic acid reagent (2 ml of a 5% solution + 98 ml of conc. H_2SO_4). After standing for 15 min and cooling to 20°, the absorption is determined at 560–580 nm. Concn. is determined from a standard curve. A second curve is obtained by determining the free HCHO in beers to which various additions of the aldehyde had been made. In this way the total content can be estimated from the level in the distillate with an error of ± 12% at concn. > 1 mg/l. Measurement of free HCHO cannot be used to detect its addition during production of malt or beer and this can only be inferred where very low anthocyanogen levels are detected. J. B. WOOF.

Determination of copper and iron in beer. H. Weyh, W. Hagen and Un Hua Pek (*Brauwissenschaft*, 1968, **21**, 472–479).—The Cu and Fe contents of German beers seem to have decreased during the past 5 to 10 years. From results of comparative determinations by a number of methods it is concluded that even today no exact method exists for the determination of Cu and Fe in beer, because colour reagent added directly to the beer does not react quant. To determine Cu quant., it is suggested that the samples are ashed between 560° and 580°; in the case of Fe, a vigorous wet digestion is recommended. (20 references.) I. DICKINSON.

Methods of measuring beer foam—A review. C. Kremkow (*Mschr. Brau.*, 1969, **22**, 53–56).—A review is given of methods used to study both head formation and its retention. The procedures may be grouped according to the method of producing the head, either by blowing up with CO_2, by shaking or by free fall. (55 references.) J. B. WOOF.

Continuous fermentation of must. C. S. Ough and M. A. Amerine (*Mitt. Klosterneuburg Rebe u. Wein Obstb. u. Früchteverwert.*, 1968, **18**, 428–439).—A fully controlled three-stage system is described which is suitable for the production of ordinary and, especially, dessert wines. (27 references.) P. S. ARUP.

Microbiological control of closed-tank fermentation of sparkling wine by means of 2,3,5-triphenyltetrazolium chloride (TTC). M. Edelényi (*Mitt. Klosterneuburg Rebe u. Wein Obstb. u. Früchteverwert.*, 1969, **19**, 17–24).—Samples of the fermenting wine containing 10^6–10^8 cells per ml are taken at intervals and centrifuged; the separated cells are then incubated with a phosphate buffer at pH 7·8, containing glucose (20 g/l) and TTC (0·05%), for periods varying from 24 h for 10^6–10^7 cells/ml to 4 h for 10^7–10^8 cells/ml. After incubation, the reaction is stopped with AcOH, and the reduced TTC is extracted into light petroleum and determined by spectrophotometry at 510 nm in comparison with a calibration graph. The accuracy is ± 0·8–2·7%. (15 references.) P. S. ARUP.

Technique of wine production in small containers (microvinification.) H. Becker (*Mitt. Klosterneuburg Rebe u. Wein Obstb. u. Früchteverwert.*, 1968, **18**, 421–427).—N_2 was bubbled through the must contained in 30-l closed carboys. The process showed favourable results as regards prevention of oxidation and of undesirable microbial infections (e.g., deliberate infection with *Candida mycoderma*) and on the redox potential. P. S. ARUP.

Formation of sulphurous acid and hydrogen sulphide during wine fermentation. K. Mayer and G. Pause (*Mitt. Geb. Lebensmittelunters. u. Hyg.*, 1968, **59**, 387–392).—In laboratory and pilot plant experiments on the fermentation of grape must with small additions of K_2SO_4, alone or with S, four out of ten yeasts produced appreciable amounts of SO_2; two yeasts produced 70 and 152 mg of SO_2 per l, respectively. The production of H_2S was slight for all the yeasts. (13 references.) P. S. ARUP.

Use of laboratory analytical results for grading of wines. B. Weger (*Mitt. Klosterneuburg Rebe u. Wein Obstb. u. Früchteverwert.*, 1968, **18**, 441–442).—Organoleptic tests may vary according to the size of the vessels used for fermentation or maturation. No analytical differences were found between samples of a must that had been fermented in glass vessels varying in capacity (1–50 l) or in a 700-l wooden vessel. No analytical changes in the various samples were observed after maturing for 8 months. P. S. ARUP.

Paper chromatographic examination of invertase activity in musts and wines. Z. Bártfay (*Mitt. Klosterneuburg Rebe u. Wein Obstb. u. Früchteverwert.*, 1969, **19**, 25–36).—A paper chromatographic technique is described which showed that the sucrose, whether solid or in solution, was rapidly hydrolysed by the invertase activity of the must. When this activity was stopped, the inversion by the yeast enzymes was comparatively very slow, and dependent on the temp., pH and the concn. of sucrose. (16 references.) P. S. ARUP.

Detection of red hybrid wines. W. Kain and H. Arndorfer (*Mitt. Klosterneuburg Rebe u. Wein Obstb. u. Früchteverwert.*, 1968, **18**, 440).—A barrel that had been used for several years for storing red hybrid wine was washed with water and then treated with a 0·6% solution of tartaric acid, containing EtOH, for 2–3 weeks. No malvidin diglycoside could be found in the solution by paper chromatography. P. S. ARUP.

Nitrate contents of Spanish wines. B. Merinero, M. J. Fernández and C. Llaguno (*Revta Agroquím. Tecnol. Aliment.*, 1968, **8**, 367–370).—Nitrate contents are reported for 100 samples of Spanish wines. 50% of the samples contained < 1 mg, 25% from

2 to 3 mg and 4% contained from 11 to 26 mg of N_2O_5 per l. It is concluded that an N_2O_5 : ash ratio > 2·5 indicates water addition. E. C. APLING.

Detection of diethyl carbonate (from addition of diethyl pyrocarbonate) in beverages. Increasing the sensitivity of detection. F. Bandion (*Mitt. Klosterneuburg Rebe u. Wein Obstb. u. Fruchteverwert.*, 1969, **19**, 37–39).—The method of Prillinger and Reinhard can be made more sensitive by extracting a 100-ml sample with 1 ml of CS_2, with the aid of centrifugation, and determining the diethyl carbonate (**I**) by g.l.c. using a support-coated open tubular column. As little as 0·05 mg of **I** (≡ 1 mg of diethyl pyrocarbonate) can be detected. P. S. ARUP.

Gas chromatographic method for determination of ethyl acetate, methanol, n-propanol, isobutanol, and isoamyl alcohol in spirits. H. Klaushofer and F. Bandion (*Mitt. Klosterneuburg Rebe u. Wein Obstb. u. Früchteverwert.*, 1968, **18**, 443–448).—To a distillate from a 100-ml sample, obtained for the determination of EtOH, are added known vol. of standard solutions in EtOH of tetrahydrofuran to act as an internal g.l.c. standard for EtOAc and MeOH, and of ethylene glycol monoethyl ether as a standard for Pr^nOH, Bu^iOH, and isopentanol. The compounds are then determined with the use of calibration graphs showing peak-dimension values in relation to standards. Total esters are determined by a saponification method. The value 'total esters minus EtOAc' is proposed as a criterion for evaluating spirits. P. S. ARUP.

Production of brewers' wort. A.P.V. Co. Ltd. (Inventor: M. G. Royston) (B.P. 1,138,801, 5.1.67).—A method for preparing brewers' wort is claimed, in which a starch adjunct is cooked in water and the resulting paste is mixed with grist and additional water to form a mash. After proteolysis the temp. is raised, saccharification effected and the wort passed to the subsequent brewing stages. In an example, a mash of rice (or maize) and water (1 : 3) is cooked at 100° for 20 min in presence of an anti-gelling enzyme fraction and then mixed with ground malt and additional water (1 : 1·75) at 46°. After proteolysis for 20 min, the temp. is raised to 67° and then to 72°, prior to filtering and further treatment. S. S. CHISSICK.

Fruits, Vegetables, etc.

Sugars, organic acids and amino acids in grapes from the Vršac vineyard. D. R. Nastić, D. L. Stanimirović, V. M. Vučković and S. G. Stanimirović (*Glasn. hem. Društ., Beogr.*, 1967, **32**, 491–504).—Glucose and fructose (and some inositol) were identified, the content of fructose predominating in all varieties; the ratio of glucose to fructose was highest in the presence of the highest content of reducing sugars. 20 amino acids were identified. Arginine and proline were the most plentiful whilst asparagine occurred only in traces. Musts known to give the best wines had the highest N content. The org. acids comprised glucuronic, malic, citric, and tartaric acids. A direct dependence of the pH on the content of free acids or on the ration of malic to tartaric acid was not observed. (38 references.) (From English summary.) P. S. ARUP.

Spectrofluorometric determination of anthocyanosides [of grapes of hybrid vines]. 3,5-Diglycoside of 3,5,7-trihydroxy-2-(3,5-dimethoxy-4-hydroxyphenyl)benzopyran. S. M. Ristić and J. M. Baranac (*Glasn. hem. Društ., Beogr.*, 1967, **32**, 137–152).—The emission value of the anionic form (malvidin) of the glycoside (in concn. 5×10^{-5} M) at 600 nm in EtOH, MeOH, or Bu^iOH containing 0·01% of HCl (RG test) gave the most reliable method of determination. Similar were spectra obtained in conc. H_2SO_4 or H_3PO_4 as solvents. The emission obtained for the anionic form in 0·1 N-methanolic NaOH at 505 nm was less persistent. The neutral (pseudobasic) form showed no fluorescence. (11 references.) (From French summary.) P. S. ARUP.

Effect of roasting on the stability of peanut [groundnut] proteins. N. J. Neucere, R. L. Ory and W. B. Carney (*J. agric. Fd Chem.*, 1969, **17**, 25–28).—The nuts were roasted for 1 h at 145°, after which the husks were removed and the cotyledons homogenised. The changes were investigated by column chromatography on DEAE-cellulose, polyacrylamide gel-electrophoresis, immuno-electrophoresis and ultracentrifugation. The solubility of the proteins in phosphate buffer was more than halved. The chief protein, α-arachin, increased in electrophoretic mobility, but kept its antigenic structure. Sedimentation analysis showed that both dissociation and association occurred. (20 references.) P. S. ARUP.

Peanut [groundnut] properties and their significance for sealed cold storage. O. Myklestad (*Fd Technol., Champaign*, 1968, **22**, 1565–1570).—In an experimental sealed cooled-wall silo (~40 in × 16 in), the temp. of the shelled nuts could be reduced by 11° within 10 h. When compressed in a food press, the deformation of the nuts obeyed the Nutting stress–strain–time equation. Respiration of the nuts in sealed storage was too slight to be of importance. P. S. ARUP.

Identification of a volatile component in soyabeans that contributes to the raw bean flavour. L. R. Mattick and D. B. Hand (*J. agric. Fd Chem.*, 1969, **17**, 15–17).—The substance was obtained by vac. distillation of the beans (previously ground with water) at < 40° in a stream of N_2, and cold trapping the distillate. G.l.c. and mass spectrometry identified the substance as ethyl vinyl ketone. P. S. ARUP.

Influence of various acidities and pasteurising temperatures on the keeping quality of fresh-packed dill pickles. R. J. Monroe, J. L. Etchells, J. C. Pacilio, A. F. Borg, D. H. Wallace, M. P. Rogers, L. J. Turney and E. S. Schoene (*Fd Technol., Champaign*, 1969, **23**, 71–77).—Satisfactory preservation of the cucumber pickles in 32-oz jars was achieved by heating to 160–170°F in HOAc with an equilibrated acidity ≥ 0·6%; the temp. was that measured inside the product placed in the coldest part of the steam-pasteuriser during treatment. Increases in the temp. and concn. of AcOH caused textural defects. The optimum time for reaching the final temp was 45 min, and the optimum packing density was for the cucumbers to occupy ~65% of the jar-space. (24 references.) P. S. ARUP.

Transient temperature distribution in whole sweet-potato roots during immersion heating. I. Thermal diffusivity of sweet-potatoes. J. I. Wadsworth and J. J. Spadaro (*Fd Technol., Champaign*, 1969, **23**, 219–223).—Temp. distributions were measured by thermocouple probes in different positions in the potatoes, immersed in water baths at 55, 70, 80 and 90°. An equation was developed from the time–temp. curves from which the thermal diffusivity (α) could be calculated. The increase in α with rising temp. became very rapid at 65–74°, and α decreased from 74 to 90°. The increase was presumably due to starch gelatinisation, and the decrease to the softening and separation of the starch cells, increased intercellular space, and enzymic degradation of the starch. (14 references.) P. S. ARUP.

Non-alcoholic beverages

Process technology in the manufacture of beverages with special regard to microbiological processes. K. Wucherpfennig (*Chemie-Ingr-Tech.*, 1969, **41**, 358).—Technical operations described include pressing of fruit, separation of the juice, its pasteurisation, evaporation with distillation of the aroma substances and the filling of the soft drinks. The maintenance of sterile conditions in each operation is stressed. M. SULZBACHER.

Changes in volatile flavour constituents of canned single-strength orange juice as influenced by storage temperature. K. S. Rymal, R. W. Wolford, E. M. Ahmed and R. A. Dennison (*Fd Technol., Champaign*, 1968, **22**, 1592–1595).—In comparison with juice that had been stored for 27 months at 4°, samples after storage at 27° contained less *d*-limonene and linalool but more furfuraldehyde and α-terpineol, as determined by the g.l.c. of volatiles obtained by low-temp. distillation and extraction of the distillate with CH_2Cl_2. P. S. ARUP.

Detection of adulterations in citrus juices. XII. Characteristics of the neutralisation curve and their variation as a consequence of adulterations. E. Primo Yúfera and J. Royo Iranzo (*Revta Agroquím. Tecnol. Aliment.*, 1968, **8**, 352–359).—Characteristics of the potentiometric titration curves are reported for 30 samples of orange juice and 25 samples of lemon juice of various maturity indexes. Max. slope values ($\triangle pH/\triangle ml$) were between 2·0 and 2·5 for orange juice and between 4·4 and 6·0 for lemon juice (previously diluted 1 : 4 with water). Max. slope values were increased by admixture of juice with solutions of citric acid or citric acid and sucrose of the same acidity as the original juice. The increase was greater the higher the proportion of adulterant solution or the lower the acidity of the original juice. The slope value is proposed as a useful index of adulteration; presumptive limits would be 2·5 and 6·0 for orange juice and diluted lemon juice, respectively. Examination of 10 commercial samples showed that at least three were adulterated. E. C. APLING.

Consistency of tomato products. V. Differentiation of extractive and enzyme inhibitory aspects of the acidified hot break process. J. R. Wagner, J. C. Miers, D. W. Sanshuck and R. Becker (*Fd Technol., Champaign*, 1969, **23**, 247–250).—In the hot break

process, the acidification to pH 3·1 (or lower) can be deferred until enzyme inactivation by rapid heating has been completed. Hot macerates can then be treated with NaOH to neutralise the added HCl before pulping and finishing. The exposure of the blending and heating apparatus to strong acid is thus avoided. (15 references.) P. S. ARUP.

Preparation of [A] navel orange juice and [B] concentrate. Salada Foods Ltd. (Inventor: J. Hanson) (B.P. 1,134,279-280, 10.11.67).—A process for producing juice is claimed, in which the precursors of bitter substances are reduced to a low level and those remaining rendered inactive. The navel or core is removed, the juice squeezed out gently without excessive fibre rupture and the juice screened to remove most of the fibres. It is then [A] chilled to $\leqslant 40°F$ and maintained at such temp., or [B] treated with an enzyme high in pectinesterase- and low in pectin polygalacturonase-activity and heated to $\sim 120°F$ for ~ 1 h, then concentrated under vac. at $\sim 75°F$ and stored at 0°F. S. S. CHISSICK.

Tea, coffee, cocoa

Composition of the aroma of tea. III. Identification of two ketones related to ionones. F. Müggler-Chavan, R. Viani, J. Bricout, J. P. Marion, H. Mechtler, D. Reymond and R. H. Egli (*Helv. chim. Acta*, 1969, **52**, 549–550).—Re-chromatography of fraction 7 of tea aroma extract, the prep. of which was previously described (*ibid.*, 1966, **49**, 1763), and of fraction 11, obtained likewise, led to the isolation of two ketones, the structures of which were established by i.r. and mass spectroscopy as 2,6,6-trimethyl-2-hydroxycyclohexanone and an epoxyionone (deriv. of β-ionone). The substances were identical with synthesised specimens. (In French.) M. SULZBACHER.

Instant coffee product. Proctor & Gamble Co. (B.P. 1,136,850, 30.11.67. U.S., 30.11.66).—A blend is claimed for producing instant coffee in which the individual particles have the same size and colour variation as in roast and ground coffee. The blend consists of (*i*) $\leqslant 10\%$ by wt. of a light coloured instant coffee having a colour (Hunter Colour values) of: 'L' scale, 25–40; 'a' scale, 5–15; and 'b' scale, 5–20; (*ii*) $\leqslant 25\%$ of a dark coloured instant coffee having a colour of: 'L' scale, 18–25; 'a' scale, 5–15; and 'b' scale, 5–15; (*iii*) the balance (if any) is conventional coloured instant coffee. The (*i*) and (*ii*) portions should differ by $\leqslant 5$ units on the Hunter 'L' scale. S. S. CHISSICK.

Milk, Dairy Products, Eggs

Identification by immunoelectrophoresis of bovine milk proteins. P. Martinez-Resa, C. Alvarez-Moreno, F. Hermida and A. Chordi (*J. Dairy Sci.*, 1969, **52**, 1–7).—An immunoelectrophoretic technique was used in detecting 26 proteins in whey, casein and whole milk. The electrophoretic mobilities and some morphological and chemical characteristics of the proteins are described. (20 references.) M. O'LEARY.

Sulphydryl and disulphide groups in casein. G. M. Wallace and K. R. Aiyar (*J. Dairy Res.*, 1969, **36**, 115–123).—A study was made of the cysteine/cystine balance in whole, acid-pptd. casein. Analysis of three different casein prep. indicated the presence of cysteine and cystine in whole casein in the proportions 1 : 1 by wt. and in a mol. ratio of 2 : 1. Ordinary processing by heating at up to 5° did not affect the sulphydryl–disulphide balance of whole casein, indicating that the sulphydryl group is not highly labile. (29 references.) M. O'LEARY.

Milk-clotting activity of proteolytic enzymes. J. Ilany-Feigenbaum and A. Netzer (*J. Dairy Sci.*, 1969, **52**, 43–46).—Many proteolytic enzymes were shown to be capable of coagulating milk but without the formation of stable clots as the coagula were further digested by continuous proteolysis. Reduction of the proteolytic activity of some enzymes (ficin and melon-extracted enzymes) resulted in the production by them of stable clots with no off-flavours. (20 references.) M. O'LEARY.

Action of calf rennet and other proteolytic enzymes on κ-casein. R. C. Lawrence and L. K. Creamer (*J. Dairy Res.*, 1969, **36**, 11–20).—Normal clotting of κ-casein by calf and fungal rennets and by various other proteolytic enzymes was prevented by the presence of α_{s1}-casein or β-casein, in the absence of Ca ions. The significance of these observations is discussed with reference to the rôle of protein–protein interactions in casein coagulation by calf rennet. (20 references.) M. O'LEARY.

A micro-method for the quantitative estimation of rennets and other proteolytic enzymes. R. C. Lawrence and W. B. Sanderson (*J. Dairy Res.*, 1969, **36**, 21–29).—A description is given of the development of an agar diffusion slide assay, using a thin layer of Ca caseinate, for use as a micro-method for the quant. study of calf rennet and other proteolytic enzymes. The method is claimed to be more sensitive than the milk clotting technique and other standard methods of measuring proteolytic activity. M. O'LEARY.

Pilot plant for the removal of cationic fission products from milk III. Nutritional evaluation of the product. R. Braude, R. F. Glascock, M. J. Newport and J. W. G. Porter (*J. Dairy Res.*, 1969, **36**, 129–136).—Milk which had been passed, at pH 5·2–5·3, through an ion exchange resin, charged with a mixture of Ca, K, Na and Mg ions, was found to be unsuitable for piglets. M. O'LEARY.

Status of iodine in milk preserved with sodium metabisulphite and formaldehyde. G. K. Murthy (*J. Dairy Sci.*, 1969, **52**, 124–125).—$Na_2S_2O_5$ was shown to be unsuitable for preserving raw milk labelled with ^{131}I because of its lack of bactericidal qualities. Recoveries of 97–100% of ^{131}I were obtained from reconstituted powdered milk, labelled with ^{131}I, and preserved with HCHO during 18 days at 23–26°. Xanthine oxidase activity was not detected in the fresly reconstituted milk, indicating no protein binding of ^{131}I. Reconstituted powdered milk, labelled *in vitro* with radionuclides and preserved with HCHO, is considered to be suitable for collaborative testing of ^{131}I or other radionuclides by ion exchange methods. M. O'LEARY.

Effect of freezing milk samples on abnormal milk test results. R. B. Read, jun., A. L. Reyes and J. G. Bradshaw (*J. Dairy Sci.*, 1969, **52**, 261–262).—Freezing milk in the range −20 to −196° and storing at −20° for 3 to 28 days depressed test scores obtained by the Wisconsin mastitis test, the electronic somatic cell count and the direct microscopic somatic cell count. Catalase values were not changed significantly except for samples frozen at −78 and −196° for 3 days. M. O'LEARY.

Effect of several factors on some reconstitution characteristics of vacuum dried whole milk. W. E. Nelson (*Diss. Abstr., B*, 1968, **29**, 237).—In the manufacture of dried whole milk, the addition of surfactants improved the sinkability, and this was a phenomenon associated with the dried milk particle surface and not the fat globule interface alone. Room temp. storage of several surfactant-treated dried whole milks showed no loss of sinkability. There was no significant change in the sinkabilities of the dried milks after 90 days of storage. The effects of high pressure (8000 psi) rehomogenisation before concn. and drying, altering the physical characteristics of the milk fat used in dried whole milk manufacture by oxidising the unsaturated bonds, and the use of different kinds of fats and oils, are discussed. F. C. SUTTON.

Some constituents of *Apium leptophyllum* (D.C.) F. Muell. in relation to tainting of milk. R. J. Park and M. D. Sutherland (*Aust. J. Chem.*, 1969, **22**, 495–496).—The essential oil (0·25%) obtained by steam distillation of fresh *A. leptophyllum* (slender celery, wild carrot or carrot weed) plants consisted mainly of thymoquinol Me_2 ether (29), carvacrol Me ether (~ 9), thymol Me ether (~ 7) and 11 terpene hydrocarbons (45%). Tests (oral dosage to cows and direct addition to dairy products) showed that the aromatic ethers are unlikely to contribute to tainting in milk or butter obtained from cows ingesting *A. leptophyllum*, but ingestion of large amounts (~ 22 kg) of the weed would cause tainting originating from the terpene hydrocarbons. W. J. BAKER.

Weed taints in dairy produce. I. *Lepidium* taint. II. *Coronopus* or land cress taint in milk. R. J. Park, J. D. Armitt (II) and W. Stark (II) (*J. Dairy Res.*, 1969, **36**, 31–35; 37–46).—I. Skatole (0·5 ppm) and 0·3 ppm of indole were found to be present in *Lepidium*-tainted butterfat. Flavour evaluation tests showed that skatole was principally responsible for the flavour defect. (14 references.)

II. Butterfat from *Coronopus*-tainted milk was shown to contain 1 ppm of benzyl methyl sulphide (**I**). The flavour thresholds of **I** in milk and butter oil were 1 part in 10^8 and 1 part in 10^7, respectively. The addition of 1 part in 10^7 and 1 part in 10^6 of **I** to untainted milk and butter oil, respectively, caused the development of a flavour defect similar to *Coronopus* taint. It is concluded that **I** is a principal contributor to the *Coronopus* flavour defect. (22 references.) M. O'LEARY.

Effect of pollution of air with ozone on flavour of spray-dried milks. F. E. Kurtz, A. Tamsma, R. L. Selman and M. J. Pallansch (*J. Dairy Sci.*, 1969, **52**, 158–161).—Skim-milk powders manufactured

in air containing 32 ppb (American) of O_3 averaged 1·7 flavour points (on a 10-point scale) lower than control powders manufactured in air containing 2 ppb of O_3. Whole milk powders manufactured in air containing 32 ppb of O_3 averaged 2·9 points lower than control powders. Foaming heightened the damaging effect of O_3 on flavour quality. Raising the O_3 level to 52 ppb produced no further significant deterioration in the flavour of the powders. M. O'LEARY.

Influence of spray-drying conditions on size and size distribution of nonfat dry milk particles. H. Hayashi, D. R. Heldman and T. I. Hedrick (*J. Dairy Sci.*, 1969, **52**, 31–37).—The mean particle size (*S*) of nonfat dry milk increased with increasing total solids or decreasing pump pressure. Particle size distribution (*SD*) was similarly affected by these two variables. *S* and *SD* were not significantly affected by differences in preheat treatment of the milk (63–85°) or variations in inlet air temp. of the spray drier (121–177°). An accurate evaluation of *SD* of nonfat dry milk was shown to require measurement by at least two methods: Coulter Counter and a sieve or microscope method. (26 references.) M. O'LEARY.

Indirect micro method for milk fat determination. M. C. Ganguli, J. D. Smith and L. E. Hanson (*J. Dairy Sci.*, 1969, **52**, 126–127).—The method uses micro-haematocrit equipment, and is suitable for both cows' and rats' milk. M. O'LEARY.

Turbidimetric methods for measuring fat content of homogenised milk. U. S. Ashworth (*J. Dairy Sci.*, 1969, **52**, 262–263).—A description is given of a procedure, based on the turbidimetric dye binding method for protein determination, for measuring the fat content of homogenised milk. M. O'LEARY.

Organoleptic properties and gas chromatography patterns of steam distillates from fresh and stale milk fat. A. Tamsma, F. E. Kurtz, A. Kontson and M. J. Pallansch (*J. Dairy Sci.*, 1969, **52**, 152–157).—Descriptions are given of steam distillation and g.c. procedures for isolating the off-flavours of stale milk fat in amounts correlated with their concn. in the fat. M. O'LEARY.

Isolation and identification of 4,8,12-trimethyltridecanoic acid from butterfat. R. P. Hansen (*J. Dairy Res.*, 1969, **36**, 77–85).—A description is given of the isolation of the isoprenoid fatty acid 4,8,12-trimethyltridecanoic acid from butterfat. The acid was found to be a DD diastereoisomer and is thought to have been derived from the phytol moiety of chlorophyll. (47 references.) M. O'LEARY.

Prevention of hydrogenation flavour during trace hydrogenation of butter oil. A. K. Vasishtha, J. G. Leeder and S. S. Chang (*Fd Technol., Champaign*, 1969, **23**, 244–247).—Hydrogenations were carried out in a 2-l. Parr apparatus using Nysel flake catalyst (containing 30% Ni). The best conditions for the selective hydrogenation of the polyunsaturated glycerides without causing a hydrogenation flavour were hydrogenation at 120° with 1% of Ni (by wt. of oil) for 30 min, and at an H_2 pressure of 20 psig. P. S. ARUP.

Gas chromatographic analysis of volatiles contributing to harsh flavour in peanut butter ice cream. P. E. Swenson and J. H. Martin (*J. Dairy Sci.*, 1969, 52, 38–42).—Peanut butter was shown to be the source of two volatile compounds, isobutyraldehyde and isovaleraldehyde, which contributed to a harsh flavour which developed in peanut butter ice cream, in which the peanut butter was incorporated before pasteurisation of the mix at 71° for 30 min. The harsh flavour did not develop in ice cream to which the peanut butter was added during freezing. M. O'LEARY.

Volatile acids in the aroma of yoghurt. M. N. Turčić, D. B. Botić and V. D. Canić (*Glasn. hem. Društ., Beogr.*, 1967, **32**, 239–246).—The aromatic compounds were obtained by steam distillation and extraction of the distillate with Et_2O. G.l.c. revealed the presence of 21–25 compounds (according to the age of the sample) including acetic (with a little formic), propionic, butyric, isovaleric, caproic, caprylic and capric acids. During storage of yoghurt at room temp., the proportions of acetic and isovaleric acids increased in relation to the others. (From English summary.) P. S. ARUP.

Dextran sulphate–Toluidine blue method for the histochemical identification of lipoproteins in cheese. V. Bolcato and P. Spettoli *J. Dairy Res.*, 1969, **36**, 125–127).—The histochemical method described is based on the formation of insol. complexes between the lipoproteins and sulphated polysaccharides. (12 references.) M. O'LEARY.

Lactose, lactic acid and mineral equilibria in Cheddar cheese manufacture. J. Czulak, J. Conochie, B. J. Sutherland and H. J. M. Van Leeuwen (*J. Dairy Res.*, 1969, **36**, 93–101).—Studies during experimental small-scale manufacture of Cheddar cheese showed that lactose fermented in the curd is replaced by lactose diffusing from the whey while lactic acid diffuses from the curd to the whey at a slower rate. A rapid attainment of a high lactic acid level in the curd caused the cheese flavour to be sour and bitter and the body to be crumbly. The development of high acidity over an extended period in the whey resulted in a reduction in the buffering capacity of the curd due to an increase in lactose and a decrease in P contents. On maturing such a cheese a bitter flavour developed. (14 references.) M. O'LEARY.

Hydrolysis of fat and protein in small cheeses made under aseptic conditions. B. Reiter, Y. Sorokin, A. Pickering and A. J. Hall (*J. Dairy Res.*, 1969, **36**, 65–76).—The influence of the cow's feeding regime on the composition of cheese fat and the hydrolysis of the cheese fat and protein by bacterial and native milk enzymes was studied in Cheddar and Edam cheese made under aseptic conditions from aseptically drawn milk. Cheese from milk of pasture-fed cows contained more *FFA* than that made from milk of cows fed hay and concentrates (winter regime). Myristic and palmitic acid contents increased markedly in the milk fat of cows on the winter regime while stearic and oleic acid contents declined significantly. Milk lipase in raw milk cheese liberated more *FFA* than the weakly lipolytic lactic acid streptococci. Milk lipase was inactivated on pasteurisation at 63° for 30 min. Rennet hydrolysed some of the cheese protein to nitrogen sol. in water, in trichloroacetic acid, or in phosphotungstic acid. Milk protease survived pasteurisation and liberated amino acids in the cheese. However, the main increase in amino acid content of the cheese was due to the proteolytic activity of the lactic acid bacteria. (30 references.) M. O'LEARY.

Use of high-temperature short-time scalding in continuous curd-making. N. J. Berridge and P. G. Scurlock (*J. Dairy Res.*, 1969, **36**, 53–63).—Laboratory experiments indicated that a *HTST* scalding at 48° for 4 min may be incorporated with advantage in continuous Cheddar curd-making processes. M. O'LEARY.

Discoloration of egg albumen in hard-cooked eggs. R. C. Baker and J. Darfler (*Fd Technol., Champaign*, 1969, **23**, 77–79).—Eggs cooked for 12 min when 1 day old were less prone to browning of the whites than those cooked when a week old. Browning was promoted by keeping the cooked eggs at 60–80° and was directly proportional to time and temp. The reaction was shown to be of the Maillard type, dependent on the presence of glucose in the whites. P. S. ARUP.

Comparison of frozen, foam spray-dried, freeze-dried, and spray-dried eggs. IV. Foaming ability of whole eggs and yolks with corn [maize] syrup solids and albumen. M. E. Zabik and S. L. Brown (*Fd Technol., Champaign*, 1969, **23**, 262–266).—The relative η, surface tension and electrophoretic patterns of the reconstituted ingredients were studied. Foaming ability was evaluated by comparing the sp. gr. of the foams and of drainage. The stabilities of whole egg foams according to processing decreased in the order: frozen, foam spray- or spray-dried, and freeze-dried (the last mentioned being very low). As regards the albumens, foams from frozen, freeze-dried and spray-dried eggs were about similar, whilst those from foam spray-dried eggs were less satisfactory. Yolks from frozen eggs were unsuitable, whilst yolks from freeze-dried and foam spray-dried eggs were better for foaming than were spray-dried products. The foaming of albumen (but not of yolk) was improved by Na lauryl sulphate; the effect on the whole egg was small. (22 references.) P. S. ARUP.

Frozen concentrated milk-acidulating culture. Chas. Pfizer & Co. Inc. (Inventors: F. V. Leghorn and E. J. Tynan) (B.P. 1,134,199, 19.4.66).—A starter culture (**I**) for use in the prep. of cheese (especially Cheddar), cottage cheese and butter milk is claimed, in which the mother culture and bulk phases are eliminated and the build-up of infectious phage in the cheese plant is minimised. A commercially available **I** is propagated in a non-milk medium (formulation given) at pH 5–7 and the concn. of cells increased, e.g., by centrifugation. The concentrate is treated with a diluent (e.g., normal saline containing 10–30% glycerol by wt.) so that the cell concn. is up to 10^{11} cells per ml, and the whole is frozen. In an example the frozen culture is added to pasteurised sweet milk at 86–88° and after agitation for 1–2 h rennet extract is added. The cheese is then prepared in the known manner. S. S. CHISSICK.

Whey product manufacture. Foremost Dairies, Inc. (B.P. 1,137,228, 8.8.66. U.S., 16.8.65 and 17.1.66).—Whey is concentrated by vac. evaporation and the lactose crystals removed.

After heating at 110–120°F and removing insol. protein, etc., the liquid material is subjected to electrodialysis at pH 3·8–4·2 (sour cheese) or 4·65–5·6 (sweet cheese). In an example, high heat raw cottage cheese whey at pH 4·2 is concentrated to 54% by wt. solids and lactose removed. After centrifugation the temp. is raised to 120°F and held at this value for 30 min. The product is centrifuged and then electrodialysed at pH 4–4·5. The final pH is adjusted to 5·0 with $Ca(OH)_2$ prior to drying. S. S. CHISSICK.

Process for deacidifying milk. Prat-Daniel S.A. (B.P. 1,137,060, 7.12.67. Fr., 15.12.66).—A process is claimed for removing excess lactic acid (**I**) formed in milk during collection, storage and handling, whereby the other milk constituents and milk quality are largely unaffected. The acidic milk is contacted (*i*) with a strongly basic anion exchanger and (*ii*) with a weakly basic anion exchanger; whereby **I** is exchanged for Cl^- and then the Cl^- removed.
S. S. CHISSICK.

Edible Oils and Fats

Radiolysis of fats with emphasis on fish oil. M. F. Dubravcic (*Diss. Abstr., B*, 1968, **29**, 237).—Volatile compounds present naturally in mackerel oil as well as those produced in oil by radiolysis (0·3–6·0 Mrad at 0 and 25°) were isolated by vac. distillation and analysed by g.l.c. and mass spectrometry. Four major hydrocarbons were obtained by radiolysis of each saturated and unsaturated triglyceride. Saturated triglycerides yielded four additional major deriv. of the parent fatty acid: alkanal, alkenal and Me and Et esters. Unsaturated triglycerides yielded the two corresponding aldehydes but no Et ester. A mechanism was proposed for the formation of the major products of radiolysis of triglycerides. F. C. SUTTON.

Application of u.v. spectrophotometry in the investigation of oxidation and polymerisation of fats. B. A. J. Sedláček (*Fette Seifen AnstrMittel*, 1969, **71**, 133–138).—Samples of sunflower seed oil were heated for up to 30 h in air, in a current of air and in a current of N_2, and during this period the u.v. absorption max. was determined at 243 nm and compared with similar determined values for η, n and sp. gr. The results indicated that there was a definite relationship between these values, although no such relationship could be found between absorption max. and peroxide value or aldehyde content. (15 references.) G. R. WHALLEY.

Occurrence and removal of phenolic substances in natural fatty raw materials. K. Thewalt, A. Pastura and G. Renckhoff (*Fette Seifen AnstrMittel*, 1969, **71**, 85–88).—The total content of phenols of coconut flesh, oil, milk and shells was found to be 10^{-5} to 10^{-2} wt.-% and they were isolated by vac. steam distillation followed by an ether extraction procedure. G.l.c. and paper chromatography were used to identify the following phenols: phenol, guaiacol, *o*-, *m*- and *p*-cresol, phenyl caproate, and 2,4-, 2,5- and 2,6-xylenol. These phenols were removed by treating the oil or split fatty acids with 0·3–0·5 wt.-% of paraformaldehyde in the presence of 0·1–1·0% of dil. H_2SO_4. With dropwise addition and slow stirring, the oil at 60–70° deposited the phenols as a slimy ppt. The origin of the phenols is discussed. (11 references.)
G. R. WHALLEY.

Fluid shortening composition. Kao Soap Co. Ltd. (Inventors: N. Matsui, T. Tomita and T. Kawada) (B.P. 1,135,417, 21.11.66).—The composition is sufficiently fluid at 5° to be easily handled and has favourable baking properties. It consists of the following ingredients where the esters are of 14–22 C fatty acids (e.g., stearic): vegetable liquid triglyceride (e.g., soyabean, cottonseed, rapeseed, maize and/or kopak oils) (80–90%); solid triglyceride (e.g., one of the above oils hydrogenated or an animal fat/oil of m.p. 40–65° (0·5–4%); mono- or di-esters of glycerol and/or of sorbitan (0–3%), mono- and/or di-esters of propylene glycol (8–16%) and of lactylated glycerol (0–5%); esters of cane sugar (0–5%) and of polyoxyethylene sorbitan (0–5%); and lecithin (0·1–2%).
S. S. CHISSICK.

Improved cooking fats. Unilever Ltd. (B.P. 1,135,609, 25.5.66. U.S., 27.5.65).—A cooking oil is claimed, containing 10^2–10^3 ppm by wt. of orange, lemon or lime oil, such that the uncooked fat is not flavoured but objectionable room odours usually produced during frying are reduced. A glycerol ester of a 9–12 C γ- or δ-hydroxy aliphatic monocarboxylic acid is also added, to impart a fried butter flavour. In an example, potatoes fried in refined, bleached, deodorised soyabean oil containing 800 ppm of cold pressed lemon oil, 500 ppm of 5-hydroxydecanoyl glyceride and 900 ppm of 5-hydroxydodecanoyl glyceride, are found to have a butterlike flavour. S. S. CHISSICK.

Meat and Poultry

Determination of type of meat [by analysis of] the fatty components and substances accompanying the fats. J. Wurziger and G. Hensel (*Fette Seifen AnstrMittel*, 1969, **71**, 144–151).—The fatty acid compositions of fats derived from beef and pork as well as horse, kangaroo, hare, tiger and elephant, were determined, together with the total cholesterol contents. It is suggested that the detection of adulterants in pork and beef can be detected on the basis of the determined linoleic, linolenic and arachidonic acid contents, together with the total quantity of cholesterol. G. R. WHALLEY.

[A] **Water-soluble flavour precursors of beef. Extraction and fractionation.** [B] **Non-aqueous beef flavour components. Composition of petroleum ether-extractable intramuscular polar lipids.** A. F. Mabrouk, J. K. Jarboe and E. M. O'Connor (*J. agric. Fd Chem.*, 1969, **17**, 5–9, 10–14).—[A] A preparative scheme is described by which the solids extracted from lyophilised beef were submitted to defatting, further lyophilisation, dialysis and gel permeation chromatographic separation on esterified Sephadex columns. Qual. g.l.c. indicated the presence of methionine and/or cysteic acid in the Sephadex fractions which had the most meaty flavour. Differences in precursor content of different meat muscles could be recognised. (26 references.)

[B] The polar lipids, separated from the neutral lipids by g.l.c. on silica gel were fractionated on an anion exchange DEAE-cellulose column into ~11 components. T.l.c. of the fractions, colour reactions, and i.r. spectroscopy of the intact lipids positively identified phosphatidylcholine, lysophosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, sphyngomyelin and phosphatidylinositol; sulphatide was tentatively identified. (14 references.) P. S. ARUP.

Flavour compounds in country cured hams. D. A. Lillard and J. C. Ayres (*Fd Technol., Champaign*, 1969, **23**, 251–254).—Volatiles were isolated by steam distillation at 0·2–0·5 torr with cold trapping, and extraction of the distillate with Et_2O for g.l.c. The carbonyl compounds were isolated as their 2,4-dinitrophenylhydrazones and identified by u.v. spectroscopy and t.l.c. The free amino acids and free fatty acids were identified by g.l.c. Compounds tentatively identified included seven mono- and di-alkenals, six alcohols, six Me esters and three Et esters. The free amino and fatty acid contents varied widely, depending on the time and temp. of maturing. (28 references.) P. S. ARUP.

Microbial profiles of fresh beef. W. C. Stringer, M. E. Bilskie and H. D. Naumann (*Fd Technol., Champaign*, 1969, **23**, 97–102).—The log. of the mean counts per in^2 (using the swab technique) was 4·7 just after slaughter, 4·78 after chilling and just before shipping and 5·94 on arrival at the shop. The chief micro-organisms found were *Pseudomonas fragi*, *P. geniculata*, and (in smaller amounts) *Micrococcus luteus*. (21 references.) P. S. ARUP.

Processing of frozen meat. W. H. Tschantz (B.P. 1,139,426, 12.4.66).—Frozen meat blocks are chipped using an apparatus consisting of a support frame, a rigid knife plate on which is mounted a knife blade, and a meat guide. S. D. HUGGINS.

Fish

Quality of fish protein concentrate prepared by direct extraction of fish with various solvents. M. N. Moorjani, R. Balakrishnan Nair and N. L. Lahiry (*Fd Technol., Champaign*, 1968, **22**, 1557–1561).—Laboratory extractions were carried out using boiling 98% EtOH, Pr^iOH, or Me_2CO for the dehydration and defatting of chopped oil-sardines (*Sardinella longiceps*) or the steam-cooked, pressed and ground fish. The residual fat in the concentrates was < 1%. The quality of product from gutted fish (especially as regards colour) was better than that from the whole fish. The Me_2CO left a slight characteristic taint. Products of uniform quality were obtained with all three solvents and they were shown in rat-feeding trials to contain no toxic substances. (12 references.)
P. S. ARUP.

Holding of raw fish (red hake) in isopropyl alcohol for *FPC* [fish protein concentrate] production. D. Dubrow and O. Hammerle (*Fd Technol., Champaign*, 1969, **23**, 254–256).—After grinding, the hake, in 10-lb batches, was stirred with 91% PrOH (1 : 1 : 3), and kept at ~22° for 1, 2, 4, 7 or 11 days. All samples of the resulting *FPC* had a lipid content of 0·3–0·5% and showed small losses of N, reducing the protein content of the *FPC*, after 11 days, by 3·4%, and the yield of *FPC* by ~0·8%. The quality of the protein was not impaired, and was better than that of casein-protein.
P. S. ARUP.

Determination of isopropyl alcohol in solid fish protein concentrate by gas–liquid chromatography. P. Smith, jun. and N. L. Brown (*J. agric. Fd Chem.*, 1969, **17**, 34–37).—The residual Pr^iOH used in the prep. of fish protein concentrate is determined by heating the sample at 180° in a closed tube for 20 min to release the Pr^iOH which is then injected into the g.l.c. system. The method is applicable to the determination of similar volatile components in other solid materials. P. S. ARUP.

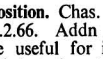
Natural and artificial radionuclides in seafoods and marine protein concentrates. T. M. Beasley, C. L. Osterberg and Y. M. Jones (*Nature, Lond.*, 1969, **221**, 1207–1209).—Analyses of these materials showed that (*i*) concn. of ^{210}Po and ^{210}Pb in fish protein concentrates ranged up to 5·8 dpm/g dry wt, and were much higher than those in beef muscle or vegetable products, (*ii*) concn. of ^{210}Po increased after hydrolytic processing but then decreased when the bone was removed, (*iii*) concn. of ^{210}Po in hydrolysed and screened products were approx. an order of magnitude higher than in common seafoods, (*iv*) average concn. of ^{210}Pb in protein concentrates was 0·45 dpm/g dry wt. The effect of human ingestion of such products on the skeletal radiation dose is discussed briefly. Although concn. of these radioisotopes are far below those constituting a radiological hazard, the continuous intake of marine protein concentrates as human food can result in the ^{210}Pb–^{210}Po pair becoming the chief source of the skeletal radiation dose. In addition, increased amounts of stable lead and other trace metals might also be ingested. (19 references.) W. J. BAKER.

Spices, Flavours, etc.

Processing and preservation of ginger by syruping under atmospheric conditions. I. Preliminary investigations of vat systems. B. I. Brown (*Fd Technol., Champaign*, 1969, **23**, 87–91).—Two vat systems for processing ginger are described, one with three vats in series and the other with six, and the characteristics of the products obtained are discussed. Optimum conditions required the use of the three-vat system at 125–135°F; temp. > 140°F caused extensive sugar inversion. (16 references.) P. S. ARUP.

Characterisation of an important aroma component of bell peppers. R. G. Buttery, R. M. Seifert, R. E. Lundin, D. G. Guadagni and L. C. Ling (*Chemy Ind.*, 1969, 490–491).—The main component of the volatile oil (1 ppm of the whole pepper) obtained by vac. steam disillation of *Capsicum annuum* var. *grossum*, Sendt is 2-methoxy-3-isobutylpyrazine. It was separated by g.l.c. on a temp.-programmed stainless steel capillary column (1000 ft) coated with silicone SF96 (100) containing 5% Igepal CO-880, and was identified by its i.r., u.v., p.m.r. and mass spectra. Larger amounts were obtained by separation on a wider silicone-packed column and then purified on a Carbowax 20-M column. The natural compound is identical with the synthetic product prepared by condensation of leucine amide with glyoxal followed by methylation of the resulting 2-hydroxy-3-isobutylpyrazine with CH_2N_2. Its odour threshold is 2 pt. per 10^{12} pt. of water. W. J. BAKER.

Flavouring composition. Chas. Pfizer & Co. Inc. (B.P. 1,135,123, 11.1.67. U.S., 11.2.66. Addn to B.P. 1,082,504).—A meatless flavouring mixture useful for imparting a beef-type flavour to foods comprises: (*i*) 1 pt. by wt. of a product of the reaction between a hexose (e.g., dextrose) or a pentose (e.g., arabinose) with cysteine (**I**) or cystine (**II**) in presence of water; (*ii*) 3–5 or 3–15 pt. by wt. of vegetable protein hydrolysate according to whether **I** or **II** is used; and (*iii*) 0·02–1·5 pt. by wt. of a 5′-ribonucleotide (e.g., inosinic and/or guanylic acids/salts). The mixture is heated at ≮ 70° for ≮ 2 h. Other, optional, ingredients (glutamic acid, glutamates, alanine, sucrose, edible fat) may be included. S. S. CHISSICK.

[*Erythro-* and *threo-*]-5-substituted-isoxazolidones and their intermediates. Takeda Chemical Industries Ltd. (B.P. 1,136,927, 23.12.65. Jap., 23.12.64. and 25.3.65).—Compounds of general formula  (**I**), $RO_2C{\cdot}CH_2{\cdot}C(X)H{\cdot}C(NHR^1)CO_2R^3$ (**II**) and $RO_2C{\cdot}CH_2{\cdot}CH{:}C(NHR^1){\cdot}CO_2R^3$ (**III**) are claimed, where R^1 is H or 1–7 C acyl, R^2 is H or 1–4 C alkyl, R and R^3 are 1–4 C alkyl and X is halogen. Prep. of **I** is from **II** by reaction with NH_2OH; prep. of **III** is from **II** by removal of HX in presence of a base. In an example, diethyl β-hydroxyglutamate·HCl in AcCl is treated with PCl_5 at ≯50° to give the corresponding **II** (X = Cl) of m.p. 112–113°. This is reacted with BzCl in aq. $NaHCO_3$ and the product treated in EtOH solution with aq. $NH_2OH{\cdot}HCl$ at −5° to give (3-oxo-isoxazolidin-5-yl)-α-benzoylaminoacetic acid. **I** include the compound tricholomic acid, a useful flavour-improving agent. S. S. CHISSICK.

Pesticides in Foods

Dichlorfluanid ('Euparen') residues on strawberries. H. V. Brewerton and M. M. Gibbs (*N.Z. Jl agric. Res.*, 1968, **11**, 784–788).—Determination of residues of *N′*-(dichlorofluoromethylthio)-*N,N*-dimethyl-*N′*-phenylsulphamide (**I**), which is used to control *Botrytis cinerea* mould on strawberries, and its main degradation product *N,N*-dimethyl-*N′*-phenylsulphamide, in four field trials under different conditions, showed that **I** has a half-life of approx. one week. E. G. BRICKELL.

Site and fate of captan residues from dipping prunes prior to commercial dehydration. T. E. Archer and J. B. Corbin (*Fd Technol., Champaign*, 1969, **23**, 235–238).—Prunes dipped in captan solution after harvesting, for the control of box-rot, lost 54–75% of the residue during air drying at 88–90° for 16 h. Dipping of the fruit, before drying, in 2% aq. KOH at 70° for 5 min and then rinsing removed all detectable residues. The processes are recommended for the improvement of quality. P. S. ARUP.

Effect of commercial and home preparative procedures on parathion and carbaryl residues in brocolli. R. P. Farrow, F. C. Lamb, E. R. Elkins, R. W. Cook, M. Kawai and A. Cortes (*J. agric. Fd Chem.*, 1969, **17**, 75–79).—Parathion, sprayed on to brocolli 3 days before harvesting, was removed in small amounts (≯ 30%) by commercial washing or steam blanching. Removals of carbaryl by washing, blanching and home freezing were 80–90%, but washing and home cooking removed only 55%. P. S. ARUP.

Degradation of chlorinated hydrocarbon pesticides in milk and butteroil by ultra-violet energy. C. F. Li and R. L. Bradley, jun. (*J. Dairy Sci.*, 1969, **52**, 27–30).—The destruction of chlorinated hydrocarbon pesticides in milk and butteroil by u.v. light of 2200–3300 Å, produced by a C arc lamp, was investigated. Under constant operating conditions, the rate of degradation was a function of the film thickness and depth of penetration of the rays. Though 96% degradation of methoxychlor in butteroil was achieved, the results generally indicated that removal of pesticides from milk and butteroil by u.v. light is unsatisfactory. (11 references.) M. O'LEARY.

Food Processing, Refrigeration

Compressed formulated products—A new concept in dehydrated foods. J. G. Fairbrother (*Fd Technol., Champaign*, 1968, **22**, 1596–1598).—Recipes and manufacturing directions are given for seven dehydrated foods prepared from various soups, vegetables and meats. Calorific values and organoleptic ratings are given for the products. (12 references.) P. S. ARUP.

Aroma concentration for dehydrated foods. J. L. Bomben, D. G. Guadagni and J. G. Harris (*Fd Technol., Champaign*, 1969, **23**, 83–86).—The Wurvac vac. stripping process was used for the prep. of aroma concentrate from apples, oranges and tomatoes. Analyses by g.l.c. and odour threshold concn. tests showed that the aroma substances had been enriched 1000-fold; losses were very small. The fresh, cold-trapped aromatic distillates could be condensed on the (largely tasteless) dehydrated fruit powders without a large increase in moisture. (11 references.) P. S. ARUP.

Thermal destruction and regeneration of enzymes in green beans and spinach purée. R. Resende, F. J. Francis and C. R. Stumbo (*Fd Technol, Champaign*, 1969, **23**, 63–66).—The heat treatment (*HTST*) of the purées (at pH 8·3) was carried out in glass capillary tubes by the method of Stern (*ibid.*, 1954, **8**, 139) with refinements to secure accurate timing. Data were obtained for the fixing of the heating times, at various temp. up to 350°F, required for the destruction (without possible regeneration) of the peroxidase content. With heating times of 1–2 sec, the required temp. would be higher than those for sterilisation. The catalase and the chlorophyll of the purées were destroyed more quickly than the peroxidase. (26 references.) P. S. ARUP.

Influence of drying conditions on quality of freeze-dried green beans. B. Lafuente, J. V. Carbonell and F. Piñaga (*Revta Agroquim. Tecnol. Aliment.*, 1968, **8**, 371–380).—Studies of the influence of SO_2 treatment, type of cut (cross- or length-wise), load density, plate temp., and sectional area of the cut samples on the drying cycle and final product quality in the freeze-drying of Blue Lake green beans are reported. SO_2 treatment improved retention of vitamin C and colour in cross-cut samples, but was less effective

for length-cut beans. With a plate temp. of 70°, drying time was about half that at 40°; the high plate temp. also favoured retention of vitamin C and colour, but organoleptic quality was considerably impaired. E. C. APLING.

Freezing chicken thighs by liquid nitrogen and sharp freezing process. K. C. Li, E. K. Heaton and J. E. Marion (*Fd Technol., Champaign*, 1969, **23**, 241–243).—Chicken thighs in nylon pouches frozen by the liquid N_2 process for 5 min at a chamber temp. of −150°F had a better colour and appearance, with less drips, and lower shearing stress values than those treated by sharp freezing at −20°F. (14 references.) P. S. ARUP.

[A] Radiation sterilisation of prefried cod and halibut patties. [B] Browning reaction in radiation-sterilised seafood products. R. O. Sinnhuber, M. K. Landers, T. C. Yu, M. Simon [A] and F. Heiligman [A] (*Fd Technol., Champaign*, 1968, **22**, 1570–1572; 1969, **23**, 224–226).—[A] Fried patties irradiated at 4·5 Mrad at room temp. and then stored at 22° for 6 months were organoleptically equal to non-irradiated patties stored at −18°, all scores being in the acceptable range. Increase of the storage time to 12 months at 22° caused significant decreases in the flavour scores of the irradiated patties. Antioxidants did not improve the flavour scores or retard the browning that was caused by irradiation.

[B] A preliminary leaching treatment with water at 77° of the fish in small pieces retarded browning during long-time storage of the patties at room temp. The addition of antioxidants (e.g., 50 ppm of Tenox), and SO_2 (500 ppm as $Na_2S_2O_5$), and possibly also $CaCl_2$ (0·4%) further contributed to the prevention of browning. (25 references.) P. S. ARUP.

Packaging

Oxygen permeability of flexible film packages for foods. E. G. Davis and R. A. Burns (*Fd Technol., Champaign*, 1969, **23**, 92–96).—Comparative tests were made on a no. of foils by the Davis test-cell methods (cf. *Aust. J. Appl. Sci.*, 1964, **15**, 309) and a package method in which sealed model packages were filled with N_2 at atm. pressure and stored in air; the gas in the stored package was sampled by means of a special apparatus (described) and analysed for O_2 by g.l.c. The two methods generally gave concordant results. Comparatively high results sometimes obtained by the package method indicated leakages due to faulty sealing or to weakness in folded edges. (13 references.) P. S. ARUP.

Packaging of cream. Central Dairy (St. Blazey) Ltd. (Inventors: P. Coulson and W. H. Smart) (B.P. 1,128,026, 26.10.65).—A desired quantity of cream is introduced into an open-mouthed transparent material (e.g., regenerated cellulose film).
E. ENOS JONES.

Miscellaneous

Nutrition, proteins, amino acids, vitamins

Composition of foodstuffs: Nutritive value tables. S. W. Souci, W. Fachmann, H. Kraut and H. Bosch; Ed. S. W. Souci and V. Hamann. 1969. (Stuttgart: Wissenschaftliche Verlagsgesellschaft m.b.H.)—A further set of tables, together with subject index and classified index, has been prepared. P. P. R.

Determining the emulsifying and emulsion stabilising capacity of protein meat additives. P. A. Inklaar and J. Fortuin (*Fd Technol., Champaign*, 1969, **23**, 103–107).—In operations, all under standard conditions, the sample is stirred with H_2O (with addition of 3% of NaCl). The stability is tested by heating the emulsion at 85° for 15 min, cooling, and then centrifuging at 3000 r.p.m. for 15 min. The vol. of oil separated is an inverse measure of the capacity to form a stable emulsion. The results obtained with soya protein and concentrate and Na caseinate were in general agreement with those obtained in sausage manufacture. (12 references.)
P. S. ARUP.

Relative activities of commercially available enzymes in hydrolysis of fish protein. M. B. Hale (*Fd Technol., Champaign*, 1969, **23**, 107–110).—Samples of enzymes, e.g., pronase (**I**), pepsin (**II**) papain (**III**), and pancreatin (**IV**), were tested on a substrate of freeze-dried haddock powder in a phosphate buffer (pH 7), containing 0·7% of BzONa, that had been steam-pasteurised. Relative enzyme activities were measured as the inverse of the wt. of the protease required to digest 60% of the standard fish powder in 24 h at the appropriate temp. The % digestion was determined by collection of the remaining insol. matter on a tared filter, washing, drying and weighing. **I** showed the greatest activity, and **II**–**IV** combined good activity with low cost. P. S. ARUP.

Potentials of leaf proteins for the preparation of concentrates from various leaf wastes in West Pakistan. A. Hussain, M. Ullah and B. Ahmad (*W. Pakistan J. agric. Res.*, 1968, **6**, 110–118).—In laboratory experiments, the leaves of 25 species were minced and pressed, and the solids were mixed with water and pressed again; the crude proteins were pptd. from the press-juices by heating to 80°, filtered off, washed, dried and powdered. Leaves of Solanaceae, Brassica and Leguminoseae yielded 40–50% of extractable proteins. Other species gave lower yields, some being < 20%. Analytical details are tabulated. (16 references.) P. S. ARUP.

Amino acid composition of gluten fractions. H. Yang and A. G. McCalla (*Can. J. Biochem.*, 1969, **47**, 379–390).—The composition of successive fractions from $MgSO_4$ pptn. of gluten dispersions in Na salicylate varied regularly, with the less sol. fractions containing less glutamic acid and proline and more arginine, lysine, aspartic acid, threonine, glycine, alanine and tryptophan than did the more sol. fractions. Ppt. produced during hydrolysis of the gluten fractions by papain also varied regularly in amino acid composition, but the proportions of individual amino acids were entirely different. Gluten must be considered as a complex combination of protein components, the no. of which cannot yet be determined. (29 references.) E. G. BRICKELL.

Vitamin A complexes. I. Influence of aluminium salts of fatty acids on stability. S. Bhattacharya (*J. Instn Chem. India*, 1969, **41**, 33–40).—Studies were made with Al salts of org. acids, Al isopropoxide (**I**) and fatty acids. High stability of vitamin A resulted from the presence of Al salts of single or mixed long chain fatty acids but it was destroyed rapidly with the salts of lower fatty acids. Half equivalent amounts of higher fatty acids with respect to **I** brought about the max. stability while individual reactants destroyed vitamin A more rapidly. (24 references.)
E. G. BRICKELL

Degradation products from ascorbic acid. J. H. Tatum, P. E. Shaw and R. E. Berry (*J. agric. Fd Chem.*, 1969, **17**, 38–40).—After an aq. solution of the acid had been boiled for 5 h, 15 compounds were separated from Et_2O extracts by g.l.c., and identified by spectrophotometric methods. Since furfural was a main product, it is probable that five of the ten furan-type compounds were formed by a benzoin-type self-condensation. Three acids, two lactones and 3-hydroxy-2-pyrone were also found. Eight of the products were identical with non-enzymic browning products found in dehydrated citrus powders. (11 references.)
P. S. ARUP.

Unclassified

Use of enzymic treatment to improve cacao paste. C. Ezguerra Larreina (*Infciones Grasas aceit.*, 1968, **6**, 168–171).—The properties of cacao pastes, untreated and treated with cellase and prolase, were compared. Cellase treatment gave the lowest η and fibre content. Prolase raised the N content, but cellase reduced it, and the highest content of reducible substances was given by cellase and the least with prolase. L. A. O'NEILL.

Continuous thin-layer fermentation. G. Gorbach (*Fette Seifen AnstrMittel*, 1969, **71**, 98–103).—The theory and practical aspects of both simple and multi-stage continuous fermentation are discussed, and the construction of a new thin-layer contact reactor is described in which, by the use of rotating rollers within fermentation tubes, nutrient layers of any desired thickness can be achieved, which are variable and dependent on the speed of rotation of the rollers. For aerobic cultures, these layers can be efficiently aerated by the introduction of a suitable current of air, which also removes metabolic substances. The rotating rollers also allow the free access of fresh nutrient and the removal of waste products. An increase in capacity is simply obtained by an increase in the no. of reactor tubes. G. R. WHALLEY.

Thermal degradation of lipids. A review. W. W. Nawar (*J. agric. Fd Chem.*, 1969, **17**, 18–21).—The mechanisms of the changes undergone during heating under non-oxidative conditions are considered. The mechanisms of the formation of lactones, Me ketones, hydrocarbons and mono- and di-carboxylic Me esters in heated fats are discussed. (28 references.) P. S. ARUP.

Gas chromatographic determination of butyric acid, and assessment of butterfat content of foods. H. Hadorn and K. Zürcher (*Mitt. Geb. Lebensmittelunters. u. Hyg.*, 1968, **59**, 369–387).—In order to avoid losses of Me esters of the fatty acids by volatilisation, the g.l.c. was carried out on the free fatty acids isolated from fats or fats extracted from foods. Saponification was carried out with 0·5 N-KOH in Pr^iOH containing 0·1 ml of H_2O per 5 ml. After evaporation of the solvent, the soaps were treated with a mixture

of CH_2Cl_2 and HCO_2H and the g.l.c. was carried out on an aliquot of the clear CH_2Cl_2 solution on an Aeropak column supporting 10% of Carbowax W, with flame-ionisation detection, and with valeric acid as internal standard. The reproducibility and accuracy were satisfactory. P. S. ARUP.

Determination of metal traces in foods and the like by means of atomic absorption spectrometry. I. Determination of silver in wine. F. Roth and E. Gilbert (*Mitt. Klosterneuburg Rebe u. Wein. Obstb. u. Früchteverwert.*, 1969, **19**, 11–16).—A 50- or 100-ml sample is evaporated down to ~20 ml and mineralised by heating with H_2SO_4 and HNO_3; the Ag is then extracted into a solution of dithizone in CCl_4. Traces down to 0·003 mg/l can be detected, and 0·03 mg/l can be determined with an accuracy of ± 15% by means of atomic absorption spectrometry. Amounts of Ag found in 10 wines were in the range 0·005–0·019 mg/l. P. S. ARUP.

Modern methods of disinfection in the foodstuffs industry. H. Edelmeyer (*Fette Seifen AnstrMittel*, 1969, **71**, 152–156).—Cleaning and disinfection in the foodstuffs industry is discussed, with special emphasis on the nature of soiled surfaces and the practical cleaning techniques. Methods described include the Venturi technique and dosing systems, as well as the use of HCHO, ethylene oxide, O_3 and u.v. radiation as sterilising agents. (27 references.)
G. R. WHALLEY.

Ecosystems of food-contact surfaces. S. K. Chaturvedi and R. B. Maxey (*Fd Technol., Champaign*, 1969, **23**, 67–70).—Interactions between milk micro-organisms and the ecological environment were examined on specially cleaned glass and stainless steel slides with respect to the milk solids, suspended solids, and residues of cleansing and disinfecting agents and of dirt in films on apparently clean surfaces. The results indicated that improvements in cleaning and sanitation techniques might be achieved after further investigations. (26 references.) P. S. ARUP.

3.—SANITATION, WATER, etc.

Limits of flammability of ethylene oxide–methyl bromide–air [fumigant] mixtures. Y. Hashiguchi and T. Ogahara (*Rep. Govt chem. ind. Res. Inst. Tokyo*, 1969, **64**, 1–5).—The explosion hazards involved in handling a mixture of ethylene oxide and methyl bromide (used as a fumigant for controlling insect pests in foodstuffs) were assessed by measuring the flammability limits in air at room temp. and atm. pressure in a spherical bomb. The ranges of flammability of all possible mixtures of the components are shown in two triangular diagrams. These limits can be calculated by Le Chatelier's law. (From English summary.) J. M. JACOBS.

Possible rôle of tyrosinase and cytochrome P-450 in metabolism of 1-naphthyl methylcarbamate (carbaryl) and phenyl methylcarbamate by houseflies. R. J. Kuhr (*J. agric. Fd Chem.*, 1969, **17**, 112–115).—Metabolism of the two carbamates by housefly microsomes required the presence of $NADPH_2$ and O_2. The metabolism was inhibited by CO, and only partly restored by light. A sol. prep. of tyrosinase, from houseflies, failed to act on carbaryl, Baygon, or phenyl methylcarbamate. Behaviour of the microsomes in the presence of selective inhibitors of tyrosinase and of cytochrome P-450 indicated that tyrosinase played little or no part in the carbamate degradation. (11 references.) P. S. ARUP.

Repellency of some phenylphenols and related compounds to houseflies. G. F. Shambaugh, J. J. Pratt, jun., A. M. Kaplan and M. R. Rogers (*J. econ. Ent.*, 1961, **61**, 1485–1487).—The most effective repellents were biphenyl and 4-chloro-2-phenylphenol. A mixture of these compounds with phenol, *o*-phenylphenol and 6-chloro-2-phenylphenol was more repellent than any single compound. C. M. HARDWICK.

Daily susceptibility of houseflies to malathion. L. Frudden and S. G. Wellso (*J. econ. Ent.*, 1968, **61**, 1692–1694).—*Musca domestica* were reared under conditions of long day (14 : 10 light : dark), short day (10 : 14 light : dark) and continuous light; when treated topically with malathion none of them exhibited a daily susceptibility rhythm. Those reared under a long day regime or under continuous light were 21% less susceptible to malathion than were those reared under short day conditions. The use of red light in the dark period did not affect results. C. M. HARDWICK.

Substituted melamines as chemosterilants of houseflies. G. C. LaBrecque, R. L. Fye, A. B. DeMilo and A. B. Bořkovec (*J. econ. Ent.*, 1968, **61**, 1621–1632).—Numerous melamines are listed, and divided into 6 groups according to the substituents attached to the 3 exocyclic N atoms of melamine. They and their salts were incorporated into the diet of flies and the effects on egg hatch, pupation and adult sterility are listed. Only one compound had an effect on oviposition; several inhibited hatch or pupation. The inconsistencies of the results are discussed.
C. M. HARDWICK.

Development of an autosterilisation technique for the housefly. R. L. Fye, G. C. LaBrecque, P. B. Morgan and M. C. Bowman (*J. econ. Ent.*, 1968, **61**, 1578–1581).—Newly emerged adults were forced to migrate through expanded polystyrene foam strands, which had been immersed in 5% tepa and dried. Strands of 10 cm or more caused complete sterility; neither sex recovered fertility within 4 weeks. Tepa contents of the flies were determined.
C. M. HARDWICK.

Laboratory methods for evaluation of toxicants for the bed bug and oriental rat flea. G. S. Burden and B. J. Smittle (*J. econ. Ent.*, 1968, **61**, 1565–1567).—Many compounds were screened for their residual effectiveness on filter paper; 105 of these caused 90% mortality after 4 weeks to *Cimex lectularius* and 119 were equally effective against *Xenopsylla cheopsis*. The more promising compounds were tested as dusts on soil against *X. cheopsis*. The LC_{95} of 11 of these are given. C. M. HARDWICK.

Degradation of fabric by American cockroach, house cricket, and striped earwig. E. L. Finley, F. G. McDermott and H. R. Gross (*J. econ. Ent.*, 1968, **61**, 1552–1557).—The insects were confined in jars with cotton (with or without resin finishes) and synthetic fabrics; half of the fabric in each case was stained with animal fat. Damage was measured visibly and by wt. loss. Visible damage by *Periplaneta americana* occurred in the order: polyester < triacetate < nylon < acrylic < viscose and acetate; damage was greater with stained fabrics. *Acheta domestica* caused increasing damage in the order: viscose < nylon < acetate < triacetate and damaged unstained more than stained fabrics. *Labidura riparia* damaged stained only nylon and to a negligible extent.
C. M. HARDWICK.

Accumulation and transfer of ^{32}P by male southern house mosquitoes. R. S. Patterson, B. J. Smittle and C. S. Lofgren (*J. econ. Ent.*, 1968, **61**, 1546–1548).—*Culex pipiens quinquefasciatus* (3rd and 4th instars) were exposed to ^{32}P in water for 48 h. Radioactivity in different parts of the reproductive organs was determined; deposition appeared to be nonselective. Radioactivity was transferred to females in the fluids that accompanied the sperm during insemination; ~0·09% of the radioactivity found in the male was transferred to the female after mating. Treatment with ^{32}P did not affect mating ability. C. M. HARDWICK.

Residues of Abate: Analysis in mosquito larvae and larvicide suspensions by flame photometric gas chromatography. M. C. Bowman, H. R. Ford, C. S. Lofgren and D. E. Weidhaas (*J. econ. Ent.*, 1968, **61**, 1586–1589).—The Melpar flame photometric detector was used together with a watercooled adaptor which permits high temp. operation. Recoveries of Abate (*O,O*-dimethyl phosphorothioate *O,O*-diester with 4,4′-thiodiphenol) from freshly prepared suspensions were ~ 100%; after standing for 16 days, < 2% of Abate was lost. Recoveries from mosquito larvae (spiked with insecticide before extraction) were ~ 99%. (14 references.)
C. M. HARDWICK.

4.—APPARATUS AND UNCLASSIFIED

Composition of tobacco paraffin hydrocarbons. I. G. Mokhnachev, V. P. Pisklov and L. A. Dulan (*Appl. Biochem. Microbiol., [USSR]*, 1967, **3**, 186–192).—In the variety discussed, normal- and iso-paraffins with 14–34 C atoms are present, those with odd numbers being more than twice as numerous as those with even numbers. Normal paraffins are more frequently met with than the iso-type and n-heptacosane and n- and iso-hentriacontane are the major components. (30 references.) C.V.

Determination of hydrogen peroxide in biological material. M. Kmínková, M. Gottwaldová and J. Hanus (*Chemy Ind.*, 1969, 519–520).—The H_2O_2 in, e.g., *A. niger* mycelium, and in the culture medium is decomposed by peroxidase in the presence of ethanolic 1% *o*-dianisidine as O_2-acceptor at pH 6·1 (0·02 M-phosphate buffer) and 22°. After 20 min the reaction is stopped by addition of conc. HCl (1 drop) and the colour intensity (proportional to H_2O_2 concn.) is measured at 420 nm. The working range is 1–15 μg/ml. W. J. BAKER.

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

AUGUST, 1969

The general arrangement of the abstracts is as follows: 1.—AGRICULTURE AND HORTICULTURE. 2.—FOOD; also appropriate Microbiological Processes; Essential Oils. 3.—SANITATION, including Water; Sewage; Atmospheric Pollution, etc. 4.—APPARATUS AND UNCLASSIFIED.

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